The Medical Nobel Institute for Biochemistry,
Department of Medical Biochemistry and Biophysics,
Karolinska Institutet,
S-171 77 Stockholm, Sweden

CHARACTERIZATION AND FUNCTION OF *ESCHERICHIA COLI* GLUTAREDOXINS

Aristi Potamitou

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To my family with endless love

ABSTRACT

Escherichia coli employs two separate pathways, driven by NADPH to reduce protein disulfides: the thioredoxin and glutaredoxin systems. Both systems function via redox active disulfides and are involved in many cellular functions including, the synthesis of DNA building blocks (by reducing the essential enzyme ribonucleotide reductase), the generation of reduced sulfur (via PAPS reductase), and the repair of oxidative damage to protein (by methionine sulfoxide reductase). This work aims in a better understanding of the two systems with emphasis on glutaredoxins. Sensitive ELISAs for the two thioredoxins (Trx1, Trx2) and the three glutaredoxins (Grx1, Grx2, Grx3) of *E. coli* were developed, and protein levels measured at different stages of growth and in different genetic backgrounds. We found that glutaredoxins were involved in antioxidant defense. Levels of all three glutaredoxins were elevated in catalase deficient strains, particularly when combined with null mutants for the thioredoxin or glutaredoxin systems. OxyR did not affect the levels of Grx2 or Grx3, as it does for Grx1, instead Grx2 levels were elevated in an *oxyR* null mutant. Grx1 and Grx2 contributed to the defense against protein carbonylation damage caused by hydrogen peroxide. Measurements of thymidine incorporation in newly synthesized DNA in relevant null mutants, showed that it is mainly Grx1 and to a lesser extent Trx1 that are involved in the reduction of deoxyribonucleotides. Grx2 was the most abundant glutaredoxin, with levels increasing at the stationary phase of growth up to one per cent of total soluble protein. Guanosine-3',5'-tetraphoshate (ppGpp) and σ^s that regulate the transcription of genes in the stationary phase of growth, affected dramatically the expression of Grx2, as did osmotic pressure and cAMP, presumably via σ^s. In accordance with the role of Grx2 as a stationary phase protein, null mutants for *grxB* were lysing at the stationary phase of growth and exhibited a distorted morphology. Null mutants for *grxB* and all three glutaredoxin genes were viable in rich and minimal media. However, a combined null mutant for all three glutaredoxins and glutathione reductase (gor^-grxA^- $grxB^-grxC^-$) was barely growing on minimal media, suggesting the possibility of a mixed disulfide mechanism for the regulation of the activity of PAPS reductase. In fact, a glutathionylated species was detected *in vivo* in poorly growing $gor^-grxA^-grxB^-grxC^-$. *In vitro* incubation of PAPS reductase with oxidized glutathione lead to the enzyme's inactivation with simultaneous formation of a mixed disulfide between glutathione and the active site Cys239. This species could be reduced and its activity restored by glutaredoxins. Reversible glutathionylation may thus regulate the activity of PAPS reductase. A novel highly abundant monothiol glutaredoxin (Grx4) was identified with maximum levels at the stationary phase of growth (750-2000 ng/mg). Expression of Grx4 is likely to be regulated by ppGpp, but not σ^s.

LIST OF PUBLICATIONS

I. Vlamis-Gardikas, A., **Potamitou**, A., Zarivach, R., Hochman, A., and Holmgren, A. (2002). Characterization of *Escherichia coli* null mutants for glutaredoxin 2. *J Biol Chem* 277, 10861-10868.

II. **Potamitou, A.,** Holmgren, A., and Vlamis-Gardikas, A. (2002). Protein levels of *Escherichia coli* thioredoxins and glutaredoxins and their relation to null mutants, growth phase, and function. *J Biol Chem* 277, 18561-18567.1.

III. **Potamitou, A.,** Neubauer, P., Holmgren, A., and Vlamis-Gardikas, A. (2002). Expression of *Escherichia coli* glutaredoxin 2 is mainly regulated by ppGpp and sigmaS. *J Biol Chem 277*, 17775-17780.

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LIST OF ABBREVIATIONS

Ahp	Alkylhydroperoxide
Ars	Arsenate reductase
cAMP	Cyclic adenosine monophosphate
CD	Circular dichroism
CRP	cAMP receptor protein
Dsb	Disulfide bond promoting enzyme
DTT	Dithiothreitol
ELISA	Enzyme immunosorbent assay
FAD	flavin adenine dinucleotide
GPX	Glutathione peroxidase
GR	Glutathione reductase
Grx	Glutaredoxin
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-S-transferase
HED	β-hydroxyethyl disulfide
HIV	Human immunodeficiency virus
HP	Hydroperoxidase/Catalase
MscL	Mechanosensitive channel L (large)
MSR	Methionine sulfoxide reductase
NAC	N-acetylcysteine
NADPH	Nicotinamide adenine dinucleotide phosphate
NF1	Nuclear factor 1
NF-κB	Nuclear factor kappa B
NMR	Nuclear magnetic resonance
PAPS	3´-phosphoadenylylsulfate
PDI	Protein disulfide isomerase
ppGpp	Guanosine-3',5'-tetraphosphate
Prx	Peroxiredoxin
RpoS or σ^s	*rpoS*-encoded sigma factor S
RR	Ribonucleotide reductase
SH	Thiol
SOD	Superoxide dismutase
SOR	Superoxide reductase
S-S, S_2	Disulfide bond
Trx	Thioredoxin
TrxR	Thioredoxin reductase
TSA	Thiol-specific antioxidant protein
Wt	Wild type

1 INTRODUCTION

1.1 THE THIOREDOXIN SUPERFAMILY

Reduction and oxidation of disulfide bonds can be mediated by a variety of thiol-redox enzymes that contain an active site with the sequence motif CXXC. These proteins may perform fast and reversible thiol-disulfide exchanges between their active site cysteines and cysteines of their disulfide substrates. The pathways of the thioredoxin and glutaredoxin systems are responsible for the reduction of intracellular disulfides *in vivo*. Thioredoxins and glutaredoxins are ubiquitous proteins with a number of isoforms in different species, and may regulate many biological functions.

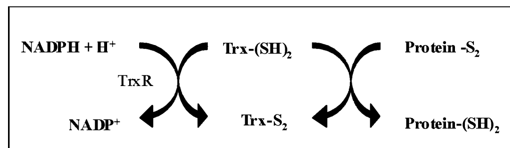


Fig 1. **General mechanism of the thioredoxin system**. Thioredoxin (Trx) is reduced by thioredoxin reductase and NADPH. Reduced Trx then reduces disulfides in a number of proteins, like RR and PAPS reductase.

Thioredoxin 1 (Trx1) of *E. coli* was originally discovered in 1964, as a dithiol cofactor for ribonucleotide reductase (RR), in the synthesis of deoxycytidine diphosphate from cysteine diphosphate (Laurent, 1964). RR is an essential enzyme for the DNA synthesis, thus for cell survival. *E. coli* contains a second thioredoxin (Trx2) that can also function as a reductant of RR (Miranda-Vizuete et al., 1997). Thioredoxins reduce their substrates employing a dithiol mechanism provided by an active site of two redox-active cysteines separated by two other amino acids (CGPC) in a coupled system with NADPH and thioredoxin reductase (Fig. 1).

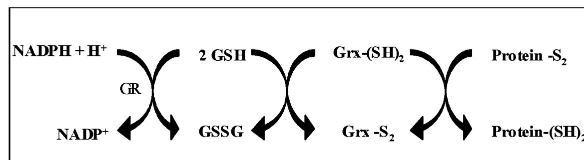


Fig 2. **General mechanism of the glutaredoxin system**. In the glutaredoxin system, electrons are transferred from NADPH, to glutathione reductase, glutathione and finally to the glutaredoxins.

In 1976, glutaredoxin was identified as a second hydrogen donor system for RR, in a mutant lacking thioredoxin (Holmgren, 1976). The other two glutaredoxins, glutaredoxin 2 (Grx2, encoded by *grxB*) and glutaredoxin 3 (Grx3, 9 kDa, encoded by *grxC*) were purified from an *E. coli* null mutant for Grx1 and Trx1 (Åslund et al.,

1994). Grx3 has only 5 % of the catalytical activity of Grx1 as a disulfide reductant for RR, and Grx2 lacks such activity altogether. Glutaredoxins use the dithiol mechanism and an additional monothiol mechanism with GSH in solution serving as the other thiol (Fig. 2) (Vlamis-Gardikas and Holmgren, 2002).

Thioredoxins and glutaredoxins have general thiol reductase activity including the reduction of 3′-phosphoadenylylsulfate (PAPS) reductase (Gonzalez Porque et al., 1970; Tsang and Schiff, 1978) and methionine sulfoxide reductase. PAPS reductase is the key enzyme for the reduction of sulfate to sulfite, while methionine sulfoxide reductase reduces methionine sulfoxide to methionine (Holmgren, 1989).

While the thioredoxin and glutaredoxin systems are responsible for maintaining a reducing cytosol, the periplasmic space is rather oxidizing and favors disulfide bond formation (Fig 3). This is upheld via the thiol oxidant DsbA and the disulfide bond isomerase DsbC, which belong to the thioredoxin structural superfamily (Bardwell et al., 1991; Missiakas et al., 1994).

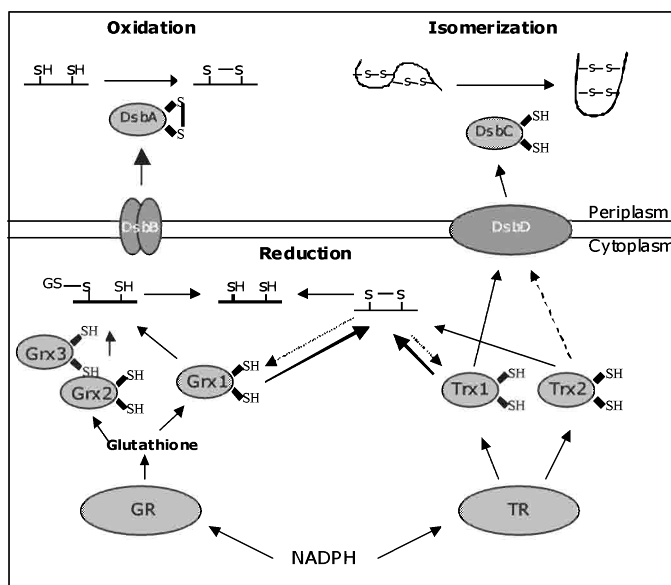


Fig 3. **Schematic overview of thiol redox reactions in *Escherichia coli*.** Reductions, oxidations and isomerizations of disulfide bonds are mediated through a variety of thiol-redox enzymes.

The active sites of thioredoxin and glutaredoxin (the CXXC motif), have been found later in a number of redox active enzymes. These include T4 glutaredoxin (Sjöberg and Holmgren, 1972), protein disulfide isomerase (PDI) (Edman et al., 1985),

DsbA (Bardwell et al., 1991) and NrdH (Jordan et al., 1997). The thioredoxin fold was identified in 1975 (Holmgren et al., 1975), from the crystal structure of *E. coli* thioredoxin 1 and is a common structural characteristic of the enzymes mentioned above. The thioredoxin fold is also present in glutathione S-transferases, and glutathione peroxidases (Epp et al., 1983; Reinemer et al., 1991).

1.2 THE THIOREDOXIN FOLD

The thioredoxin fold (crystal structure of *E. coli* Trx1 in 1975 (Holmgren et al., 1975)) is comprised of a central core of five β-sheets (three parallel and two anti parallel strands), surrounded by four α-helices. The well-conserved active site sequence (WCGPC) is located at a protrusion turn between β2 and α2 (residue 29 to 37), and is exposed to the solvent. Although the sequence homology of thioredoxin among different species can vary to a great extent (27-69 %), the fold is conserved. Apart from the well-conserved active site sequence, residues Asp26, Ala29, Trp31, Asp61, Pro76 and Gly92 are important for thioredoxin function and are thus also well conserved in *E. coli* Trx1 (Eklund et al., 1991; Martin, 1995).

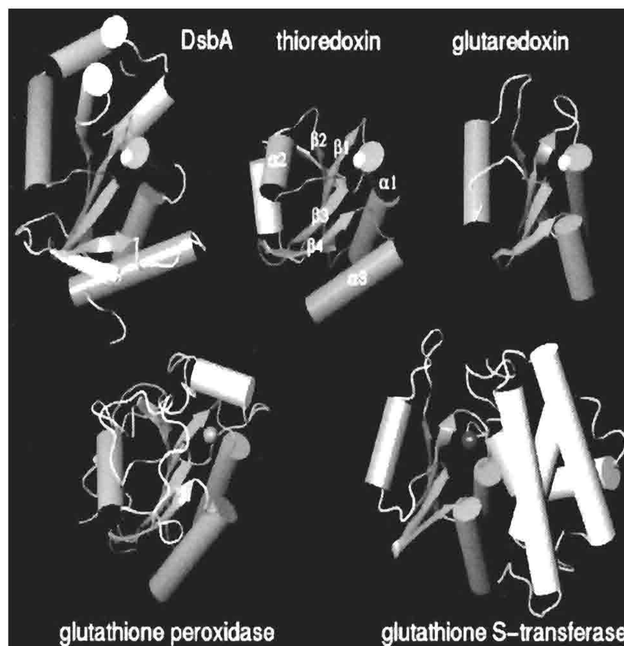


Fig. 4. **Three-dimensional structures of five thioredoxin-fold proteins** (figure from (Martin, 1995)).

Several other proteins share the common structural fold with thioredoxin, and thus belong to the thioredoxin superfamily (Fig. 4). The sequence identity within the superfamily may be low, but they all share the basic thioredoxin fold, characterised by four to five beta sheets flanked by three to four α-helices. Proteins of the thioredoxin fold superfamily, include glutaredoxin, protein disulfide isomerase, DsbA, NrdH, glutathione S-transferase and glutathione peroxidase (Eklund et al., 1992; Epp et al., 1983; Kemmink et al., 1996; Martin et al., 1993; Reinemer et al., 1991; Sodano et al., 1991; Stehr et al., 2001). The four first enzymes catalyse thiol disulfide oxidoreduction and contain the active site CXXC. The enzymatic reaction takes place at the N-terminal part of the protein. Glutathione S-transferase (GST) and glutathione peroxidase (GPx) on the other hand, interact with the cysteine containing substrate GSH in a similar manner as glutaredoxin. Glutaredoxin, GSTs and GPxs also have a GSH binding site. The only other factor that unites these proteins apart from the common thioredoxin fold is thus the cysteine chemistry, since all of these proteins interact with substrates containing a thiol or a disulfide group.

In contrast to the relatively low homologies among different thioredoxins, glutaredoxins exhibit rather high amino acid sequence homology particularly in the area of the active site (Fig. 5). The three-dimensional structures of a number of glutaredoxins from different species, including bacteriophage T4, vaccinia Grx1, *E. coli* Grx1, Grx2, Grx3, pig Grx1, and human Grx1 have been determined (Eklund et al., 1992; Katti et al., 1995; Kelley and Bushweller, 1998; Nordstrand et al., 1999; Sodano et al., 1991; Sun et al., 1998; Xia et al., 2001). These studies have revealed three characteristic regions within these dithiol glutaredoxins. First is the active site CXXC motif (usually CPYC), second the hydrophobic area and finally a well-defined binding site for glutathione. The latter involves two intermolecular backbone-backbone hydrogen bonds forming an antiparallel intermolecular β-bridge between the protein and glutathione (Bushweller et al., 1994; Nordstrand et al., 1999). In *E. coli* Grx3, these interactions involve residue Lys8, Tyr13, Arg16, Arg40, Arg49, Asp66 and Asp67 (Fig. 5). The binding of GSH for *E. coli* Grx1 and Grx3 is overall very similar. Three-dimensional structures of the oxidized and reduced form have been determined and compared for *E. coli* Grx1, and revealed that the solvent accessible surface of the conserved hydrophobic area increases upon reduction. This could perhaps favour interactions with substrate proteins. After reduction of the substrate, the decrease of the hydrophobic interaction area of the now oxidized glutaredoxin could facilitate the release of the substrate (Xia et al., 1992).

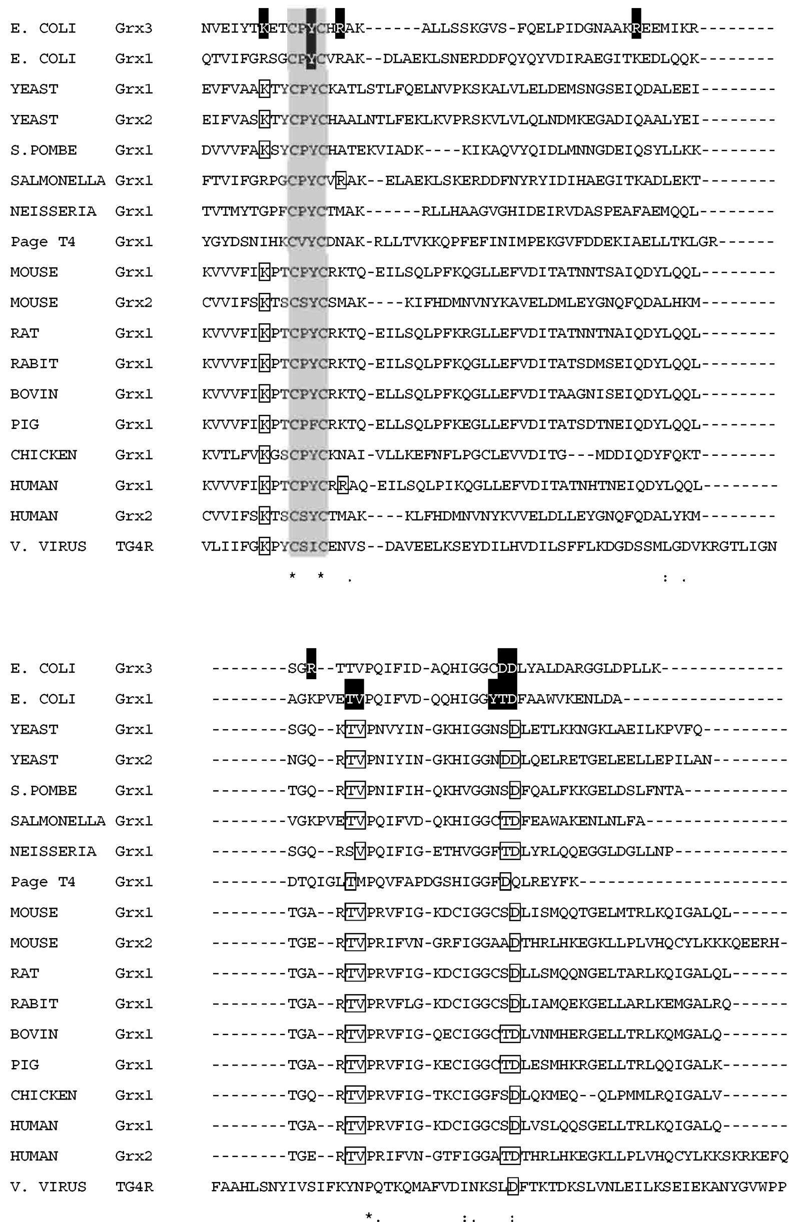


Fig. 5. **Sequence alignment of glutaredoxins.** Glutaredoxin active sites are printed on a grey background. Residues involved in the binding of glutathione in *E. coli* Grx1 and Grx3 are marked with a black box (Nordstrand et al., 1999; Xia et al., 1992), while the predicted corresponding residues from the other glutaredoxins are marked with a white box.

1.3 THE ACTIVE SITE

Disulfides may affect protein structure and activity. Generally disulfide bonds stabilize protein structure (e.g. BSA), while biological activity of proteins may also be affected (e.g. OxyR). In some oxidoreductases, formation and reduction of disulfides is essential for enzymatic activity as part of a catalytic mechanism (e.g. ribonucleotide reductase and PAPS reductase).

TABLE I
The active site of proteins belonging to the thioredoxin superfamily, catalyzing thiol-disulfide interchanges.

Protein / protein family	**Active site sequence**
DsbA	CPHC
DsbB	CVLC
DsbC	CGYC
DsbD	CVAC
DsbE	CPTC
DsbG	CPYC
CcmH	CPKC
Glutaredoxin	CPYC
NrdH	CVQC
PDI	CGHC
Thioredoxin	CGPC
Human Grx2	CSYC
Monothiol glutaredoxins	CGFS

The formation, isomerization and reduction of disulfide bonds may be catalysed by the members of the thioredoxin family, which are pivotal for thiol-disulfide metabolism. They all have a conserved active site that consists of two cysteine residues, separated by two intervening amino acids, the CXXC motif (Table I). These enzymes are capable of both forming and reducing disulfide bonds, according to the biological need. Oxidation and reduction of the disulfides of the substrate is mediated by thiol disulfide exchange with the reduced active site cysteines of the enzyme.

To evaluate the reducing capacities of different enzymes it is necessary to determine their correct redox potential (Table II). Redox potential is a measure of the reducing capacity of a redox couple and is expressed as units of millivolts (mV). The standard state redox potential E^o for a protein can be calculated from the equilibrium constant of the redox reaction involving a reference with known redox potential, using the Nernst equation ($\Delta E = RT/(nF)\ln Keq$). E^o is the redox potential under standard conditions, 1M and 25 ^oC. Considerable experimental and theoretical studies have been

made based on the effect of the residues (X_1X_2) between the active site cysteines (CX_1X_2C) on their standard state redox potential. The first mutagenesis study made, demonstrated that redox potential of an enzyme of the thioredoxin structural superfamily is affected by the residues X_1 and X_2. For example, a thioredoxin mutant with the active site of protein disulfide isomerase (PDI) is 35 mV more oxidizing compare to the wild type protein, although is still far from that of wild type PDI (Krause et al., 1991). A DsbA mutant containing the thioredoxin active site, resulted in a lowering of the E^o with 90 mV, making it a 1000 fold better reductant (30 mV corresponds to an equilibrium constant of 10 for a two electron thiol-disulfide interchange reaction), although again far from the value of the native thioredoxin (Huber-Wunderlich and Glockshuber, 1998). In comparison, a thioredoxin variant with the DsbA active site becomes a 200 fold better oxidant (Mossner et al., 1998).

TABLE II
Redox potential of the thioredoxin superfamily of proteins

Protein /substrate	**Redox potential (E^o) (mV)**
DTT	-320
Trx1	-270
Grx1	-233
Grx3	-198
DsbA	-122
DsbB	-271 and -284
DsbC	-130
DsbD α	-229
DsbD γ	-241
DsbG	-125
NrdH	-248

Other studies have shown that there is a close relationship between the redox potential and the pKa value of the N-terminal active site cysteine. A low pKa value of the N-terminal cysteine tends to stabilize the reduced form of the protein relative to the oxidized form, resulting in an increased redox potential. The thioredoxin family of proteins displays a lower pKa value for the N-terminal cysteine compared to a typical thiol (pKa of 8.7). The reactive cysteine for Trx1 for instance is 6.3 and only 3.5 for DsbA (Kallis and Holmgren, 1980; Nelson and Creighton, 1994). The extremely low pKa of the nucleophilic cysteine of DsbA stabilizes the reduced state of the protein making it an excellent disulfide bond donor. It is not only the redox potential that is affected by the pKa value of the involved thiols, but it is also the rate of thiol-disulfide interchange.

1.4 RIBONUCLEOTIDE REDUCTASE

Ribonucleotide reductase is a ubiquitous cytosolic enzyme that catalyses the *de novo* synthesis of deoxyribonucleotides required for DNA replication and repair in all living organisms (For more detailed reviews on the subject see (Eklund et al., 2001; Jordan and Reichard, 1998; Poole et al., 2002; Reichard, 2002; Sjöberg and Sahlin, 2002)). It is an essential enzyme for the DNA synthesis and thus also for the survival of all living organisms. The enzyme reduces all four main ribonucleotides to the corresponding deoxyribonucleotides. The synthesis occurs through the reduction of the 2´-hydroxyl group of the sugar moiety of ribonucleotide to hydrogen. The direction of the reaction, from ribonucleotides to deoxyribonucleotides is consistent with the concept of an RNA world preceding today's DNA based life. The need of DNA as a life molecule required ribonucleotide reductase.

Ribonucleotide reductase is composed of two units, a radical generator and a reductase. The radical generator produces and stores a radical, which, as a first step of the reaction, is used to oxidize the substrate to a radical form. Interestingly, the radical generator is not the same for all ribonucleotide reductases whereas the reductase component is fairly similar. A specific feature of ribonucleotide reduction is that a single protein reduces all four common ribonucleotides and that allosteric effects regulate substrate specificity (Eklund et al., 2001; Reichard, 2002; Sjöberg, 1997).

Three classes of ribonucleotide reductases have been described with great differences in their primary and quaternary structures. They all have a highly similar allosteric regulation of their substrate specificity (Brown and Reichard, 1969; Sjöberg, 1997). The classification is based on the radical generating mechanism and on the structural differences (Table III). It is further believed that all classes aroused and evolved from a common origin, a primitive "ur-reductase" (Reichard, 1997; Reichard, 1993).

Class I enzymes are strictly aerobic and can be subdivided into two further subclasses (Ia and Ib). The enzyme consists of two subunits (α and β), both in dimeric form. The first, large subunit contains a substrate binding site, two allosteric control sites and a sulfhydryl group. The second, smaller subunit participates in the catalysis of the reaction by generating a free radical in each of its chains. The radical (in this case the tyrosyl radical), is stable for many days (Fontecave et al., 1992). *E. coli* contains the genetic information for two different class I RRases. One of them, RR1a (or NrdAB, encoded by the *nrdAB* operon), is essential for growth in the presence of oxygen, whereas the other one, RR1b (or NrdEF, encoded by the separate *nrdEF* operon), is normally not fully functional (Jordan et al., 1996). NrdAB and NrdEF have limited sequence similarity and differ in their allosteric regulation (Eliasson et al., 1996; Jordan et al., 1994). Trx1 and Trx2 are hydrogen donors for NrdAB but not for NrdEF (Jordan

et al., 1994; Miranda-Vizuete et al., 1997). NrdH is more specific for NrdEF rather than for NrdAB, whereas in the case for Grx1 it is the opposite (Jordan et al., 1997). The expression of *nrdAB* genes is cell cycle-regulated and increases when DNA synthesis is inhibited.

Although no class II enzymes have been found in *E. coli*, they are common among aerobic and anaerobic eubacteria (Panagou et al., 1972; Tsai and Hogenkamp, 1980). The enzyme consists of a single protein, which may form monomers or dimers, depending on the organism. From a functional point of view, class II enzymes are functionally equivalent to the large protein (α) of Class I. They thus lack the small subunit and contain no stable radical. The radical in these enzymes, is of a transient type and is generated by adenosylcobalamin during catalysis (Licht et al., 1996).

Class III enzymes are strictly anaerobic and become inactivated by oxygen. They are homodimers (encoded by *nrdD*), with a stable oxygen sensitive glycyl radical. A small protein, termed activase (encoded by *nrdG*) is closely linked to the first homodimer unit, and contains an Fe/S cluster that together with S-adenosyl-L.methionine (AdoMet) can generate the stable glycyl radical (Ollagnier et al., 1996; Sun et al., 1996; Tamarit et al., 1999).

TABLE III

Summary of the three classes of ribonucleotide reductase

	Class Ia	**Class Ib**	**Class II**	**Class III**
Distribution	Bacteria Eukaryotes	Bacteria	Bacteria	Bacteria
Operation	Aerobic	Aerobic	Aerobic & Anaerobic	Anaerobic
Structure	$\alpha_2\beta_2$	$\alpha_2\beta_2$	α or α_2	$\alpha_2 + \beta_2$
Genes	*nrdAB*	*nrdEF*	*(nrdJ)*	*nrdDG*
Metal-center	Fe-O-Fe	Fe-O-Fe Mn-O-Mn	Co	4Fe-4S
Stable Radical	Tyrosyl	Tyrosyl	None	Glycyl
Catalytic Radical	Thiyl	Thiyl	Thiyl	Thiyl
Reductant	Thioredoxin Glutaredoxin	NrdH Glutaredoxin	Thioredoxin	Formate

1.5 3´-PHOSPHOADENYLYLSULFATE (PAPS) REDUCTASE

Sulfur is an essential element in all living organisms with many different functions. The reduced form is found in amino acids, lipoic acid and iron-sulfur clusters, while the oxidized form is a constituent of polysaccharides and lipids. Protrophic bacteria or fungi mainly use inorganic sulfate as the only supply of sulfur for

the biosynthesis of their amino acids and essential cofactors. Most of the sulfur in living organisms is present in the form of thiols.

Inorganic sulfate is reduced and incorporated into bioorganic compounds in a pathway named assimilatory sulfate reduction. This occurs in five enzymatic steps. First, sulfate is activated to adenylylsulfate (APS) and 3´-phosphoadenylylsulfate (PAPS) by ATP sulfurylase and APS kinase. The activated sulfate, PAPS, is then reduced to sulfite by PAPS reductase and sulfite is reduced to sulfide by sulfite reductase. Finally, sulfide is incorporated into an active amino acid receptor, *O*-acetylserine (OAS), O-acetylhomoserine or O-succunylhomoserine, to form cysteine or homocysteine. PAPS reductase catalyzes the first reductive step in the assimilatory sulfate pathway, which is present in plants, fungi, yeast and a wide range of eubacteria.

PAPS reductase is a homodimer, with each subunit having a molecular mass of 28 kDa, and a single cysteine involved in the catalysis of the substrate. The active site, a highly conserved ECGLH-motif is located near the C-terminus (Berendt et al., 1995). PAPS reductase is reduced by thioredoxin or glutaredoxin. In 1970 Gonzalez Porque´ *et a*l. (Gonzalez Porque et al., 1970) identified thioredoxin as the reductant of PAPS reductase while investigating methionine sulfoxide and sulfate reduction in yeast. Tsang and Schiff later found glutaredoxin as an alternative reductant in a thioredoxin null mutant for *E. coli* (Tsang and Schiff, 1978). In bacteria and fungi, a single glutaredoxin or thioredoxin is essential for sulfate reduction and thus for the viability of the organism. In *E. coli*, it is Trx1 or Grx1 that is essential for the sulfate reduction (Lillig et al., 1999). Kinetic data, together with the three-dimensional structure, suggest a ping-pong mechanism for the reduction PAPS reductase (Berendt et al., 1995; Krone et al., 1991; Savage et al., 1997). The oxidized form is reduced by thioredoxin or glutaredoxin to a stable reduced isoform via a disulfide mechanism, which cannot be replaced by a monothiol counterpart as GSH or the dithiol DTT. The reduced form is reoxidized by PAPS to give free sulfite and 3´-5´-bis-phosphate (PAP).

1.6 THE THIOREDOXIN SYSTEM OF *ESCHERICHIA COLI*

The thioredoxin system of *E. coli* consists of NADPH, thioredoxin reductase (TrxR, encoded by *trxB*) and thioredoxins 1 and 2 (Trx1 and Trx2, encoded by *trxA* and *trxC*, respectively). Thioredoxins are kept in a reduced state by thioredoxin reductase, which in turn is constantly being supplied with electrons from NADPH. The thioredoxin system plays a central role in the response to oxidative stress, and is involved among other things in the regulation of DNA synthesis, gene transcription, cell growth and apoptosis (reviewed by (Arner and Holmgren, 2000; Williams et al., 2000)).

1.6.1 Thioredoxin reductase

Thioredoxin reductase (TrxR) was first discovered in *E. coli* as the enzyme that allowed NADPH to act as hydrogen donor for ribonucleotide reductase in the presence of *E. coli* thioredoxin (Moore et al., 1964; Thelander, 1967). TrxR is present in all living cells and belongs to the pyridine nucleotide-disulfide oxidoreductase family of flavoenzymes. Two types of TrxR have been characterized, and both function as homodimers. Each monomer possesses a FAD-binding domain, a NADPH-binding site and an active site comprising a redox active disulfide. However, the amino acid sequences and catalytic mechanisms of the two types differ greatly with 20 % sequence identity. The first type is present in prokaryotes, arachea and some lower eukaryotes, with each subunit having a molecular mass of 35 kDa. The second type is found in higher eukaryotes, is much larger, with a subunit of 55 kDa and is homologous to glutathione reductase (GR), lipoamide dehydrogenase (LipD), mercuric reductase (MerR) and trypanothione reductase (TryR). The "low M_w TrxRs" of the first type, are more related to alkyl hydroperoxide reductase (AhpF). The "high M_w TrxRs" have a redox active center in the FAD binding domain and an elongated C-terminal domain (the interface domain), which are both absent in the low M_w TrxRs. The interface domain is involved in dimerization and catalysis and contains a conserved redox active center, in mammals characterized by Gly-Cys-SeCys-Gly, where SeCys is selenosysteine (Arscott et al., 1997; Gladyshev et al., 1996; Zhong et al., 1998). Selenium is essential for the activity of the mammalian TrxR, where a mutation from SeCys to Cys leads to a 100-fold lower k_{cat} (Zhong et al., 1998; Zhong and Holmgren, 2000). Structural analysis of the *E. coli* TrxR revealed a mechanism of transferring electrons that differs greatly from that of the TrxR of higher eukaryotes and is unique for the microbial TrxRs. The mechanism involves significant conformational changes in order to transfer the reducing equivalents from the NADPH-binding domain to the disulfide-containing domain. Once the protein bound FAD is reduced, the pyridine nucleotide-containing moiety has to rotate approximately 67° before the electrons can be transferred to the domain containing the redox active disulfide (Lennon et al., 1999; Lennon et al., 2000). This might be the reason why the *E. coli* TrxR has been shown to have very narrow substrate specificity, and only reduces thioredoxins, in contrast to the mammalian TrxRs, which has a much broader range of substrates (Arner and Holmgren, 2000; Williams et al., 2000). Recently a novel protein, thioredoxin glutathione reductase (TGR), was identified that consists of TrxR fused with a glutaredoxin domain in the N-terminus (Sun et al., 2001). In mammals the glutaredoxin domain consists of a monothiol active site, while in other organisms it has a dithiol active site (Agorio et al., 2003). These proteins can reduce Trx, GSSG, and a GSH-linked disulfide *in vitro*.

Null mutants in *E. coli* of *trxB* show a high increase in disulfide bond formation, and are not viable when combined with genes encoding for either glutathione reductase (*gor*) or glutathione synthetase (*gshA*) unless supplemented with a reducing agent like dithiothreitol (DTT) (Prinz et al., 1997). This shows that the presence of the thioredoxin or glutaredoxin systems is essential for the survival of the cell. The highly oxidizing environment of the *trxB* null mutant may be reversed when combined with additional null mutants for either of the two thioredoxins. This is due to the reversal role of the thioredoxins in the absence of TrxR. In cells lacking TrxR, oxidized thioredoxin accumulates in the cytoplasm and acts as an oxidant, catalyzing the formation of disulfide bonds (Stewart et al., 1998). Furthermore, the oxidizing properties of thioredoxins are dependent on the redox potential of the active site of the enzyme. Overexpression of Trx1 mutants, differing in the sequence of the dipeptide within their active sites, showed remarkable differences of the cytosolic redox state. For example overexpression of a more oxidizing form, like the one with the glutaredoxin active site, had higher content of disulfide bond forming proteins in the cytosol compared with overexpression of the wt Trx1 (Bessette et al., 1999). Null mutants for *trxB* have also been used to study the bacterial type I secretion pathway, which does not require a periplasmic intermediate of the secreted protein. A disulfide bond containing single-chain Fv (scFv) antibody fragment was used for this end, and it was found that the *trxB* null mutant inhibited the secretion of this protein fragment. It was thus suggested that premature cytoplasmic oxidation of proteins may interfere with the secretion process (Fernandez and de Lorenzo, 2001).

1.6.2 Thioredoxins

Thioredoxins are small redox active proteins present in all living organisms. They have been isolated and characterized from a wide variety of prokaryotic and eukaryotic cells. Thioredoxins reduce their substrates employing a dithiol mechanism provided by two redox-active cysteines separated by two other amino acids, usually CGPC.

1.6.2.1 Thioredoxin 1

Trx1 is a small 12-kDa heat stable protein that displays many different functions mainly via redox-mediated processes. It has a well-described structure (thioredoxin fold) and conserved redox active sequence (WCGPC). Trx1 was initially discovered as a potent reductant of ribonucleotide reductase, the essential enzyme for the reduction of ribonucleotides to deoxyribonucleotides during *E. coli* aerobic growth (Laurent, 1964). The sequence, including the active site motif, for Trx1 was determined four years later (Holmgren, 1968). Trx1 can also reduce 3´-phosphoadenylsulfate (PAPS) reductase and methionine sulfoxide reductase (Gonzalez Porque et al., 1970; Tsang and Schiff, 1976). PAPS reductase is the key enzyme for the reduction of sulfate

to sulfite, while methionine sulfoxide reductase reduces methionine sulfoxide to methionine (Holmgren, 1989). In addition, Trx1 can act as an efficient antioxidant to protect cells against oxidative stress (Holmgren, 2000). Trx1 has been shown *in vitro* to regulate the activity of the transcription factor OxyR. OxyR is active in its oxidized form. By reducing OxyR, Trx1 thus deactivates OxyR. Further studies showed that *E. coli* Grx1 is likely to be the preferred reductant of OxyR *in vivo*, since a *trxA* null mutant showed an identical profile as the wild type strain in the response to hydrogen peroxide, and did not affect the OxyR activity (Zheng et al., 1998). The levels of Trx1 increase at early stationary phase (Lunn et al., 1984). It was later shown that the expression is induced in the stationary phase of growth by ppGpp in an RpoS independent manner (Lim et al., 2000).

As mentioned above, Trx1 is found in its oxidized form in null mutants for *trxB*. It was also suggested that this oxidized form might promote disulfide bond formation *in vivo*. Attaching a signal sequence to Trx1 resulted in transferring of Trx1 to the periplasm. Trx1 was able to promote disulfide bonds in the periplasm and could partially complement a *dsbA* minus strain, which is defective in disulfide bond formation. It was thus concluded that the redox function of Trx1 is dependent on the redox environment in which it is localized (Debarbieux and Beckwith, 1998). While wild type Trx1 could only partially complement DsbA, more oxidizing variants, could completely replace DsbA deficiency (Jonda et al., 1999). One such variant of Trx1 is the one containing the DsbA active site, which exhibited kinetics indistinguishable from those of DsbA itself. DsbB performs the reoxidation of the thioredoxin variants. A complex between DsbB and Trx1 has also been isolated confirming the finding that DsbB is responsible for the reoxidation of Trx1 in the periplasm (Debarbieux and Beckwith, 2000). Levels of *E. coli* Trx1 are elevated upon osmotic upshock (Scharf et al., 1998). In a shift back to low osmolarity conditions, Trx1 is secreted via the mechanosensitive channel (MscL) to the periplasm (Ajouz et al., 1998).

Apart from being a general disulfide reductant, Trx1 performs other functions. It is for instant essential for the life cycle of the bacteriophages T7, M13 and f1 (Chamberlin, 1974; Lim et al., 1985; Russel and Model, 1985). Trx1 is essential for the assembly of the filamentous phages in f1 and M13. The phage T7 incorporates *E. coli* thioredoxin as an essential subunit of its DNA polymerase. T7 DNA polymerase is a stable noncovalent 1:1 complex between thioredoxin and the T7 gene 5 protein. Bound reduced thioredoxin enhances the activity of the polymerase more than 100-fold by increasing processivity. For Trx1 to be active in this role, it is required in its reduced form (Holmgren et al., 1978).

1.6.2.2 Thioredoxin 2

More recently a novel thioredoxin (Trx2) was cloned with 139 amino acid residues and with a calculated molecular mass of 15.5 kDa (Miranda-Vizuete et al., 1997). Trx1 and Trx2 show 38 % sequence identity, with the greatest difference being an extension of Trx2 in the amino terminus of 32 amino acids. The elongated N-terminal includes a $CXXCX_{16}CXXC$ motif. Trx2 is less heat stable than Trx1, probably due to the elongated N-terminal part of Trx2. The activity of Trx1 and a truncated form of Trx2 was not affected after 5 min incubation at 85 °C, wile the full-length Trx2 lost 40 % of the insulin-reducing activity after 5 min at 85 °C. On the other hand, the two thioredoxins have similar steady state kinetic properties with TrxR. Trx2 can also reduce RR1a and PAPS reductase *in vitro* (Lillig et al., 1999; Miranda-Vizuete et al., 1997), but not as efficiently as Trx1. It is unlikely though, that Trx2 is an *in vivo* reductant of PAPS reductase as combined null mutants for *trxA* and *grxA* cannot grow on minimal media containing sulfate (Russel and Holmgren, 1988). Trx2 is able though to support growth under aerobic conditions in a triple mutant lacking Trx1, Grx1 and Grx3 the three known electron donors for RR1a (NrdAB), confirming the *in vivo* function of Trx2 as a reductant for RR1a. Similar to Trx1, Trx2 was not a hydrogen donor for RR1b (NrdEF). By overexpressing Trx2 to similar levels as Trx1, Trx2 could complement growth defects caused in the *trxA* mutant and could function as a hydrogen donor for PAPS reductase *in vivo*, and reduce the periplasmic disulfide isomerase DsbC. Contrary to Trx1, Trx2 can not act as an electron donor for methionine sulfoxide reductase and cannot restore a functional T7 phage infection cycle (Ritz et al., 2000). It has been suggested that cysteine residues other than those of the active site of Trx2 may regulate its activity. This was postulated from the finding that the activity of Trx2 was increased after preincubation with DTT. A truncated form lacking the N-terminal part of the protein containing the extra cysteines was further found to be insensitive to DTT treatment, all pointing to a regulatory function of the extra cysteines. Immunoblotting analysis showed that Trx2 is a cytosolic protein, mainly localized in the peripheral part of the cytosol at the inner surface of the cytoplasmic membrane. In contrast to Trx1, the transcriptional regulator OxyR that regulates major responses to oxidative stress positively affects the transcription of *trxC* (Ritz et al., 2000). OxyR binds to the *trxC* promoter region located immediately upstream of the –35 sequence, which corresponds to a consensus sequence for OxyR binding. Levels of Trx2 are also 20-fold upregulated after treatment with hydrogen peroxide in an OxyR dependent manner. The N-terminal cysteines of Trx2 are not oxidized *in vivo*, even when the cytosol is very oxidizing. Moreover, Trx2 may be induced under oxidative conditions, because in contrast to Trx1, which becomes fully oxidized, Trx2 remains active as a thiol reductant even under conditions of severe oxidative stress (Ritz and Beckwith, 2001).

1.6.3 NrdH

NrdH belongs to a "new" class of small redox proteins (glutaredoxin-like proteins) and has been found in several organisms including *E. coli*. NrdH proteins have especially been found in organisms lacking glutathione. Although NrdH has a glutaredoxin-like amino acid sequence, it behaves functionally like a thioredoxin, since it lacks activity with GSH but is a substrate for TrxR. Furthermore, it has a low redox potential and it is active in the insulin assay, both characteristic features of thioredoxins. NrdH differs in its intervening residues from the glutaredoxins (typically CPYC) and the thioredoxins (typically CGPC), having valine and glutamine (CVQC) instead. The *in vivo* function of NrdH is not completely clear. It can act as the functional electron donor for class Ib ribonucleotide reductase (RR1b)(NrdEF) and is part of an *nrdHIEF* operon (Jordan et al., 1997; Jordan et al., 1996; Torrents et al., 2000). As Trx1, NrdH can function as a subunit for bacteriophage T7 polymerase, thus supporting growth of the bacteriophage in a *trxA* null mutant (Ritz and Beckwith, 2001). Recently, *E. coli nrdhHIEF* mRNA levels were found over 20-fold increased, in cells treated with an oxidant (Monje-Casas et al., 2001). Transcription of the aerobic ribonucleotide reductase 1b from the *nrdHIEF* operon is also increased over 100-fold in strains lacking both Trx1 and Grx1. The regulatory mechanism for the transcription of *nrdHIEF* is unknown, but it has been shown not to be through a global regulator like, RpoS, cAMP, Fis, OxyR, SoxRS or RecA (Monje-Casas et al., 2001).

The crystal structure of recombinant *E. coli* NrdH has been determined (Stehr et al., 2001). The protein belongs to the thioredoxin superfamily and is structurally most similar to *E. coli* Grx3 and phage T4 glutaredoxin. The GSH binding sites present in glutaredoxins are generally not conserved in NrdH, and no glutathione–binding cleft is identified. NrdH contains instead a wide hydrophobic pocket at the surface, similar to that of thioredoxins (Stehr et al., 2001).

1.7 THE GLUTAREDOXIN SYSTEM OF *ESCHERICHIA COLI*

The glutaredoxin system was first discovered in 1976 in a null mutant for thioredoxin 1 in *E. coli*. In the glutaredoxin system, electrons are transferred from NADPH, to glutathione reductase (GR), glutathione (GSH) and finally to the three glutaredoxins (Grx1, Grx2 and Grx3) (Åslund et al., 1994; Holmgren, 1976).

1.7.1 Glutathione

As early as 1888, the French scientist de Rey-Pahlade, described a "sulfur-loving" compound called philothion. The English scientist Fredrick Gowland Hopkins later renamed this compound to glutathione in 1921. Glutathione (L-gammaglutamyl-L-cysteinylglycine) is a tri-peptide of the amino acids glutamic acid, cysteine, and glycine. The primary biological function of glutathione is to act as a non-enzymatic

reducing agent to reduce cysteine thiols on the surface of proteins. Glutathione may prevent oxidative stress in most cells and helps in the trapping of free radicals that can damage DNA, RNA and proteins (for a detailed review se (Penninckx and Elskens, 1993)).

Glutathione synthesis occurs in two closely linked and enzymatically controlled reactions that both utilize ATP and draw on non-essential amino acids as substrates (Meister, 1995). First, cysteine and glutamate are combined (by the enzyme γ-glutamylcysteinyl synthetase), with availability of cysteine usually being the rate-limiting factor. Cysteine is generated from the essential amino acid methionine, from the degradation or turnover of proteins. The buildup of GSH may inhibit the enzyme's activity, thereby helping to ensure homeostatic control over GSH synthesis. The second GSH synthesis reaction combines γ-glutamylcysteine with glycine to generate GSH (catalyzed by glutathione synthetase) (Apontoweil and Berends, 1975).

Glutathione exists in two forms: The "reduced glutathione" tripeptide is conventionally called glutathione and abbreviated GSH; the oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide or GSSG. Glutathione is present inside cells mainly in its reduced GSH form. Oxidized glutathione can be reduced by glutathione reductase. Glutathione often attains millimolar levels inside cells (up to 10 mM), which makes it one of the most highly concentrated intracellular antioxidants. The GSSG/GSH ratio may also be a sensitive indicator of oxidative stress, as well as for cell growth, development and signaling (Schafer and Buettner, 2001). A change in redox equilibrium has been shown to favor the formation of protein disulfides and glutathione mixed disulfides with proteins (Cotgreave et al., 2002). Formation of protein disulfides or GSH mixed disulfides may protect irreversible damage of cysteine residues. In addition, it may be an important mechanism in regulating an enzyme's activity.

The first *E. coli* mutants found to be defective in the synthesis of glutathione, were simultaneously isolated and characterized by two different groups (Apontoweil and Berends, 1975; Fuchs and Warner, 1975). The isolated mutant was the gene encoding for glutathione synthase, *gshB*. The *gshB* mutant contained no detectable glutathione, but contained an increased pool of γ-glutamylcysteine. The *gshB* cells were very sensitive to diamide (Hibberd et al., 1978). At the same time, the *gshA* mutant was isolated, and was found to have increased sensitivity to a large number of chemical agents (Apontoweil and Berends, 1975). A *gshA* deficient strain is also more sensitive to osmosis. A *gshA* null mutant is not able to grow above 1.4 osM, and it grows more slowly at moderate osmolarities, and has a longer lag phase during osmotic upshock (McLaggan et al., 1990). It was therefore suggested that this mutant might have altered membrane permeability. The effect of GSH on the superoxide-sensitive [4Fe-4S]-containing aconitase has also been investigated. It was found that the

aconitase activity was approximately 25 % lower in the *gshA* strain compared to the parental strain growing on either glucose or succinate (Gardner and Fridovich, 1993).

Even though GSH can reduce hydrogen peroxide and organic peroxides to form water and alcohol, respectively, mutations in the *gshA* gene show normal resistance to X-irradiation and hydrogen peroxide (Apontoweil and Berends, 1975; Greenberg and Demple, 1986). Depletion of GSH causes hypersensitivity of *E. coli* to acroline, the structurally simplest α, β-unsaturated aldehyde derived from degradation of lipid peroxide. It was shown also that GSH chemically reacted with acroline in vitro, and thus reduced its toxicity. It was further demonstrated that acroline inactivated glutathione reductase, which was followed by a depletion of GSH (Nunoshiba and Yamamoto, 1999).

1.7.2 Glutathione reductase

Glutathione reductase is a flavoprotein catalysing the reduction of glutathione disulfide (GSSG) to glutathione (GSH). The enzyme is a dimer with 50 kDa subunits. The association of the two subunits are arranged in a "head to tail" manner. Each subunit consists of an $NADP^+$-binding domain, flavin adenine dinucleotide (FAD)-binding domain and an interface domain. Glutathione is bound to the FAD domain of one of the subunits and to the interface domain in the other subunit. The electrons from NADPH are transferred to the FAD, then to the two cysteine residues and finally to the oxidized glutathione. Surprisingly, the ratio of reduced to oxidized glutathione does not appear to change significantly in mutants that lack glutathione reductase (Tuggle and Fuchs, 1985).

The gene encoding glutathione reductase in *S. typhimurium*, *gor*, is regulated by the transcription factor OxyR (Christman et al., 1985). The levels of GR increased four-fold in an OxyR overproducing strain. A couple of years later, a similar response was found in *E. coli*, were an increase of GR was seen, in an OxyR dependent manner, after treatment of cells with hydrogen peroxide (Storz and Tartaglia, 1992). In 1995 it was further reported that the transcription of the *gor* gene was positively regulated by the transcription factor σ^s at the stationary phase of growth (Becker-Hapak and Eisenstark, 1995). The finding was a result from the knowledge that the levels of GSH in *E. coli* rise six-fold in the stationary phase, although the levels of enzymes responsible for GSH synthesis remain constant throughout growth (Apontoweil and Berends, 1975; Loewen, 1979). Both RpoS and OxyR can thus regulate GR. In a null mutant for *rpoSoxyR*, the levels of GR were significantly lower than in the oxyR single mutant, and could be restored by overproduction of a plasmid encoding *rpoS* (Becker-Hapak and Eisenstark, 1995). In the same report, absence of GR increased resistance of *E. coli* to certain oxidants, such as hydrogen peroxide, methyl viologen and *N*-

ethylmaleimide (NEM). No effect was detected between the *gor* strain and the parental strain after treatment with menandione and cumene hydroperoxide.

1.7.3 Glutaredoxins

Glutaredoxins are general thiol-disulfide oxidoreductases (Holmgren, 1979a; Holmgren, 1979b) that can reduce protein disulfides, by a dithiol mechanism or mixed disulfides forming between oxidized GSH (GSSG) and proteins or low molecular weight thiols, by a dithiol and/or monothiol mechanism (Bushweller et al., 1992). Today, *E. coli* has three well-characterized glutaredoxins (Grx1, Grx2, and Grx3, encoded by *grxA*, *grxB* and *grxC* respectively). Because of the strong preference of glutaredoxins for glutathione mixed disulfides, they have been proposed to participate in an enzyme's regulation, particularly under oxidative conditions (Gilbert, 1984; Gravina and Mieyal, 1993).

Glutaredoxins are now known to exist in most living organisms, including prokaryotes (e.g. *E. coli*), plants (e.g. rice, spinach, poplar, *A. thaliana*), viruses (e.g. bacteriophage T4, vaccinia, HIV), and eukaryotes (e.g. yeast, *P. falciparum*, rabbit, calf, pig, and human) ((Holmgren, 1976))((Cho et al., 1998; Minakuchi et al., 1994; Morell et al., 1995; Rouhier et al., 2002))((Ahn and Moss, 1992; Davis et al., 1997; Eklund et al., 1992))((Gan et al., 1990; Hopper et al., 1989; Lundberg et al., 2001; Luthman et al., 1979; Padilla et al., 1995; Rahlfs et al., 2001; Yang and Wells, 1991)).

1.7.3.1 Glutaredoxin 1

Glutaredoxin 1 was the first glutaredoxin discovered, and identified as the second donor for ribonucleotide reductase, in a mutant lacking thioredoxin 1 in *E. coli* (Holmgren, 1976). Even though thioredoxin is more abundant in the cell (10 μM) compared to glutaredoxin (1 μM), glutaredoxin has a 10-fold lower K_m for ribonucleotide reductase (Holmgren, 1979; Holmgren et al., 1978). Grx1 is an alternate electron donor to thioredoxin for the reduction of PAPS reductase (Tsang, 1981; Tsang and Schiff, 1978). By overexpressing Grx1 to similar levels as Trx1, Grx1 could also rescue the growth defects of a *trxAmetE* null mutant and could reduce methionine sulfoxide reductase (Stewart et al., 1998).

Grx1 has 85 amino acid residues including the active site sequence CPYC, and a molecular weight of 10 kDa. Thermodynamic stability experiments showed that oxidized and reduced Grx1 are very similar in stability. In heat-induced denaturation, monitored by circular dichroism (CD) the T_m were 55 and 57 ^oC for oxidized and reduced respectively. In GuHCl denaturation, the midpoint denaturation concentrations were 2M for both oxidized and reduced form. This differs greatly from the thioredoxin in *E. coli*, were the oxidized form is far more stable than the reduced

(Sandberg et al., 1991). The three-dimensional structure of Grx1 has been determined by NMR spectroscopy, in both its oxidized and reduced form (for more details see above). The three-dimensional structure is similar to that of Trx1, although the sequence identity is very low. In addition, the glutaredoxins contains a glutathione-binding site, which is not present in the thioredoxins (Sodano et al., 1991; Sodano et al., 1991; Xia et al., 1992). *E. coli* Grx1 has close homologues in most living organisms (Martin, 1995).

The first null mutant for Grx1was constructed in 1988, and showed no significant phenotype (Russel and Holmgren, 1988). The combined null mutant for *trxAgrxA* was viable in rich media, but was not viable in minimal media, unless supplemented with reduced cysteine or glutathione. The finding led to the conclusion that either Trx1 or Grx1 is essential for the reduction of PAPS reductase. However, the null mutant for *trxAgrxA* maintained deoxyribonucleotide synthesis, with an increase of ribonucleotide reductase activity of up to 23-fold, implying that a third hydrogen donor must exist in the cell (later found to be Trx2 and Grx3) (Miranda-Vizuete et al., 1994; Russel et al., 1990). Lack of thioredoxin or glutaredoxin would lead to an increase level of one or the other (as determined by ELISA), to maintain a balance supply of deoxyribonucleotides. Grx1 was 10-fold induced in the absence of thioredoxin reductase (Höög et al., 1983). An extremely high (70-fold) increase was observed for Grx1 in null mutants for *gshAtrxA* (Miranda-Vizuete et al., 1996).

Grx1 is induced by hydrogen peroxide in an oxyR-dependent fashion (Tao, 1997). The transcriptional regulator OxyR is sensitive to oxidation and activates the expression of antioxidant genes (there among Grx1) in response to hydrogen peroxide (for more details about OxyR see below). Grx1 catalyzes the reduction of OxyR *in vivo*, and since OxyR regulates Grx1, the response is auto regulated (Åslund et al., 1999; Zheng et al., 1998).

1.7.3.2 Glutaredoxin 2

Glutaredoxin 2 (Grx2) was purified from an *E. coli* null mutant lacking Trx1 and Grx1 (Åslund et al., 1994). Characterization of Grx2 showed that it was highly different from the other known glutaredoxins in terms of molecular weight, amino acid sequence and catalytic activity (Vlamis-Gardikas et al., 1997). Grx2 cannot reduce RR or PAPS reductase, but has the highest catalytic activity using the mixed disulfide between glutathione and β-hydroxyethyl disulfide as substrate with a turnover of 554 s^{-1} (HED assay) (Vlamis-Gardikas et al., 1997). *E. coli* Grx2 has close homologues in *Actinobacillus actinomycetemcomitans* (87 % amino acid identity), *Neisseria meningitidis* (58 %) and *Vibrio cholerae* (42 %), all known pathogens but it is not a 'ubiquitous' protein. In contrast to Grx1 (and Grx3), Grx2 is a much larger enzyme (24.3 kDa) with the N-terminal, residue 1-72, forming a glutaredoxin

domain, connected by an 11 residue linker to the highly helical C-terminal domain, residue 84-215 (Xia et al., 2001). The structure of Grx2 is similar to glutathione-S-transferases, although there is no obvious sequence homology. The structural similarity is interesting, since a relatively new class of mammalian GST-like protein, the single cysteine ω class, have glutathione oxidoreductase activity, rather than the glutathione-S-transferase activity.

1.7.3.3 Glutaredoxin 3

Grx3 was identified at the same time as Grx2 in the null mutant lacking Trx1 and Grx1 (Åslund et al., 1994). Grx3 had 5 per cent of the catalytic activity of Grx1 for ribonucleotide reductase, but lacked activity for PAPS reductase (Åslund et al., 1994; Lillig et al., 1999). Even though Grx3 can act as a electron donor for RR1a *in vitro*, it most probably cannot reduce RR1a *in vivo*. Triple mutants lacking Trx1, Trx2 and Grx1 are inviable and can only grow when cotransfected with a plasmid overexpressing any one of these three proteins (Stewart et al., 1998). Grx3 consists of 82 amino acid residues (10 kDa protein), with 33 % sequence identity to *E. coli* Grx1 (Åslund et al., 1996). The active site of Grx3 is positioned between residues C11 and C14. The activity of Grx3 is reduced in a Grx3H15V mutant, indicating electrostatic contributions for the stabilization of C11 (Nordstrand et al., 1999). The three-dimensional structure of Grx3 confirmed the structural analysis that Grx3 has a thioredoxin/glutaredoxin fold with a well-defined binding site. In addition, the solution structure suggested a binding site for a second glutathione (Nordstrand et al., 1999). Denaturation of Grx3 with GuHCl showed no difference in stability between the reduced and oxidized forms (Åslund et al., 1997).

TABLE IV

Regulation, sensitivity and substrates of the thioredoxin and glutaredoxin systems

Gene	Protein	Regulation	Sensitivity	Specificity
gor	Glutathione reductase	OxyR, ppGpp	H_2O_2, CHP, tBHP, diamide	Glutathione
grxA	Glutaredoxin 1	OxyR	H_2O_2	RR, PAPS reductase, OxyR, ArsC, (MSR)
grxB	Glutaredoxin 2	Acid stress	H_2O_2	ArsC
grxC	Glutaredoxin 3			(RR), ArsC
trxA	Thioredoxin 1	ppGpp, osmosis	H_2O_2	RR, PAPS reductase, MSR, (OxyR), DsbD
trxB	Thioredoxin reductase			Trx1 and Trx2
trxC	Thioredoxin 2	OxyR		RR, (PAPS reductase)

1.8 GLUTAREDOXIN FUNCTION AND REGULATION

In comparison to thioredoxins, less is known about the actual function of the glutaredoxins. Apart from PAPS reductase and ribonucleotide reductase mentioned above, glutaredoxins can participate in the reduction of ascorbate (Wells et al., 1990). The dehydroascorbate reduction activity of glutaredoxins is likely to be important for glutathione-dependent regeneration of dehydroascorbate from ascorbate (reviewed by (Meister, 1992)).

Glutaredoxins can function as efficient hydrogen donors for peroxiredoxin from poplar sieve (Rouhier et al., 2001). The reduction of peroxiredoxin is either via a dithiol or a monothiol mechanism (Rouhier et al., 2002a; Rouhier et al., 2002b).

Human Grx1 can catalyse the reduction of plasma glutathione peroxidase (Björnstedt et al., 1994). The human plasma glutathione peroxidase is an extracellular selenoenzyme that detoxifies hydroperoxides. Furthermore, the yeast dithiol glutaredoxins (Grx1 and Grx2) possessed a glutathione peroxidase activity converting hydroperoxides to the corresponding alcohols. It was proposed that the glutathione peroxidase activity of the glutaredoxins could be conjugated to GSH by glutathione-S-transferases (Collinson et al., 2002).

The mitochondrial yeast Grx5 is essential for the activity of Fe/S enzymes and null mutants for Grx5 causes iron accumulation in the cell. Thus, it has been suggested that Grx5 is part of the mitochondrial machinery involved in the synthesis and assembly of iron/sulfur centers (Rodriguez-Manzaneque et al., 2002).

Glutaredoxins are required for the reduction of arsenate reductase (ArsC) in *E. coli* (Gladysheva et al., 1994). ArsC catalyses the reduction of arsenate to the less harmful compound arsenite. ArsC has a single catalytic cysteine residue, Cys12, that can form a covalent thiolate-As(V) intermediate. Glutaredoxins are required to reduce the enzyme bound ES-As(V) intermediate to an ES-As(III) intermediate. Mutants lacking the N-terminal cysteine in the active site, leading to an inactive enzyme, could not catalyse the ArsC-As(V) reduction, while mutants lacking the C-terminal cysteine could still support the activity of ArsC (Shi et al., 1999). This finding led to the conclusion that the ArsC intermediate is not formed during the catalytic cycle but instead an ArsC-S-SG complex, which subsequently is reduced by glutaredoxins via a monothiol mechanism. From the *E. coli* glutaredoxins, Grx2 has the highest catalytic activity, in reducing arsenate reductase (Shi et al., 1999).

The redox regulation of transcription factors is important and especially during oxidative stress in several signal transduction pathways. Nuclear factor I (NFI) is for instance sensitive to oxidative inactivation due to the presence of a conserved, oxidation-sensitive cysteine residue (Cys3) within the NFI DNA-binding domain (Bandyopadhyay and Gronostajski, 1994). Cys3 forms mixed disulfides with glutathione, which decreases the DNA-binding activity of NFI. Glutaredoxin can

reduce the mixed disulfide and thus reactivate the DNA-binding activity of the oxidized, inactive NFI (Bandyopadhyay et al., 1998).

Another protein whose its biological activity is regulated via reversible glutathionylation is G-actin. The glutathionylation site has been identified as Cys374. Deglutathionylation can be efficiently catalysed by glutaredoxin, and results in a 6-fold increase in the rate of actin polymerisation (Wang et al., 2001).

Protein tyrosine phosphatases participate in the control of cell cycle and signal transduction. Glutathionylation of Cys215 of protein tyrosine phosphatase 1B gives inactive enzyme. Human glutaredoxin is able to reactivate the enzyme by reducing the glutathione mixed disulfide (Barrett et al., 1999).

Glutaredoxins are potent antioxidants against dopamine-induced oxidative stress in rat cerebral granule neurons, preventing their apoptosis by activating the binding activity of nuclear factor kappa B (NF-κB) (Daily et al., 2001a). *E. coli* Grx2 was able to penetrate into the granule neurons and exert its activity by activating NF-κB. Addition of Grx2 resulted in translocation of NF-κB from the cytoplasm to the nucleus, by promoting the phosphorylation and degradation of I-κBα. In addition, the DNA binding activity of pre-existing nucleus NF-κB was enhanced. The effect was mediated by upregulation of Ref-1, which in turn activated NF-κB. Grx2 could actually activate both the Ras/phosphoinotiside 3-kinase/Akt/NF-κB and the JNK1/2/AP1 cascades (Daily et al., 2001b).

Tyrosine hydroxylase is the initial and rate limiting enzyme in the biosynthesis of the neurotransmitter dopamine. Posttranslational modification in terms of glutathionylation of the enzyme results in a strong reduction of its catalytic activity. Glutaredoxin can fully restore enzyme activity by reducing the glutathione mixed disulfide (Borges et al., 2002). It is thus likely that the glutaredoxin system may regulate the dopamine biosynthesis under conditions of oxidative stress or drug induced toxicity.

Two glutaredoxins have been characterized in Vaccinia virus, G4L and O2L. They have a predicted thioredoxin fold and contain the redox active CXXC motif. Both enzymes are active in the classical "HED-assay", and they show dehydroascorbate reductase activity *in vitro* (Ahn and Moss, 1992; Gvakharia et al., 1996). G4L is an essential intermediate in cytoplasmic disulfide formation for virion assembly (White et al., 2002). Vaccinia virus encodes and packages its own glutaredoxins in its virus particle. This is in contrast to the immunodeficiency virus type 1 (HIV-1), which has been reported to carry the human glutaredoxin within its viral particle (Davis et al., 1997).

Glutaredoxins can catalyze the reduction of GSSG by reduced dihydrolipoamide with high efficiency (Porras et al., 2002). The lipoamide/HED activity ratio was highest for *E. coli* Grx2. These findings suggest a new role for the

glutaredoxins using reducing equivalents from the catabolic pathways, to maintain glutathione in the reduced state, without the consumption of NADPH (Porras et al., 2002).

Finally, studies of human Grx1 has shown that is it widely distributed and found in all tissues examined. However, the expression of Grx1 is especially high in tissues with high metabolic turnover (e.g. Skeletal and heart muscle, liver kidney and brain), as well as in epithelial tissue of the skin and tongue, where high number of cells undergo cell differentiation (Padilla et al., 1992; Rozell et al., 1993). Except for its wide distribution in cells, glutaredoxin has also been detected in plasma, indicating an extracellular role for human Grx1 (Nakamura et al., 1998).

The thioredoxin and glutaredoxin systems of *E. coli* can compensate for each other *in vivo* (Table V).

TABLE V

Phenotypes for null mutants of the thioredoxin and glutaredoxin systems

Genotype	**Protein**	**Comment**
trxA	Trx1	Increased sensitivity to cumene hydroperoxide, improved viability after exposure of H_2O_2
trxC	Trx2	
trxB	TrxR	Formation of disulfides in the cytosol, improved viability after exposure of cumene hydroperoxide
grxA	Grx1	Slightly increased sensitivity to diamide
grxB	Grx2	See paper I
grxC	Grx3	Slightly increased sensitivity to cumene hydroperoxide and menandione
gor	GR	Maintain GSH in a reduced state, increased sensitivity to diamide
trxAgrxA		Not viable in minimal media, growth slow under aerobic conditions, no growth defect under anaerobic conditions
gortrxA		Glutathione is maintains in a reduced state
gshAtrxA		Highly increased Grx1 levels
trxBgor		In need of a reductant to grow
trxAtrxCgrxA		Not viable under aerobic conditions

1.9 GLUTAREDOXIN ISOFORMS

The glutaredoxin family has grown during the last years, and there are today numerous isoforms known in different organisms with largely different catalytic properties. In terms of their structure and catalytic properties, glutaredoxins can now be classified in three categories (Vlamis-Gardikas and Holmgren, 2002).

The first is exemplified by the classical glutaredoxins, which are 10 kDa proteins, with the CXXC motif (usually CPYC) as their active site and with the thioredoxin/glutaredoxin fold. Grx1 and Grx3 of *E. coli*, belong to this first classical category. Both are ~10 kDa proteins with similar structure (the thioredoxin /glutaredoxin fold) and they have 33% sequence identity (Åslund et al., 1996; Bushweller et al., 1994; Martin, 1995).

The second category is structurally related to the glutathione-S-transferases, but with glutaredoxin oxidoreductase activity. Common structural characteristics are a two-domain structure, the first domain having a thioredoxin/glutaredoxin fold containing the active site residues and the second domain having a highly α-helical structure. This class of glutaredoxins is defined by *E. coli* Grx2. *E. coli* Grx2, has a three-dimensional structure, highly similar to glutathione-S-transferases (Xia et al., 2001). It only differs form the glutathione-S-transferases in that it contains the active site sequence CPYC in the glutaredoxin domain, and thus has glutaredoxin activity. Other proteins that are structurally related to this category even though they have no significant amino acid homology and only one active site cysteine, are the human θ class glutathione-S-transferase, the human glutathione-S-transferase ω 1 (GSTO1), the mouse glutathione-S-transferase θ-like stress response protein (p28), and the human chloride intracellular channel 1 (CLIC1) (Board et al., 2000; Harrop et al., 2001; Kodym et al., 1999; Rossjohn et al., 1998). All these proteins are detoxifying or stress response proteins.

The third category of glutaredoxins is defined by having a monothiol active site (normally CGFS). Monothiol glutaredoxins have so far been identified in yeast (yGrx3, yGrx4 and yGrx5) and man (PICOT). The yeast monothiol glutaredoxins have a protective role against oxidative stress. A mutant lacking Grx5 was very sensitive to both menandione and hydrogen peroxide and contained high amounts of carbonylated proteins compared to the parental strain. The mutant had increased sensitivity (more than 10-fold) to high concentrations of KCl. A yeast null mutant for the three-monothiol glutaredoxins was not viable suggesting that monothiol glutaredoxins are very specific for their substrates and their functions cannot be replaced by their dithiol counterparts (Rodriguez-Manzaneque et al., 1999). The human monothiol, PKC-interacting cousin of thioredoxin (PICOT), is expressed in various tissues, and when overexpressed in T-cells, it inhibits the activation of c-Jun N-terminal kinase and the transcription factors AP-1 and NF-κB (Witte et al., 2000). Monothiol glutaredoxins

have been identified in many different species, through genome databank searches (Fomenko and Gladyshev, 2002; Isakov et al., 2000).

1.10 CATALYTIC MECHANISM OF GLUTAREDOXIN

Glutaredoxins catalyze GSH-disulfide oxidoreductions usually via two redox active cysteine separated by two other amino acids (typically CPYC) (Holmgren, 1989; Holmgren and Åslund, 1995). The oxidoreductions are either dithiol reactions reducing protein disulfides or monothiol reductions of mixed disulfides with glutathione. In comparison, the structurally related thioredoxins may predominantly reduce protein disulfides.

In the dithiol reduction, the solvent exposed N-terminal cysteine of the active site sequence of the glutaredoxin, initiates a nucleophilic attack on one of the sulfur atoms of the disulfide target (Fig. 6A). This results in the formation of a mixed disulfide between the glutaredoxin and the target protein (Fig. 6B). The free second carboxy-terminal cysteine of the active site gets deprotonated and attacks the N-terminal glutaredoxin sulfur atom participating in the mixed disulfide with the target protein (Fig. 6C). As a consequence, oxidized glutaredoxin ($Grx-S_2$) and reduced target ($Prot-(SH)_2$) are generated (Fig. 6D).

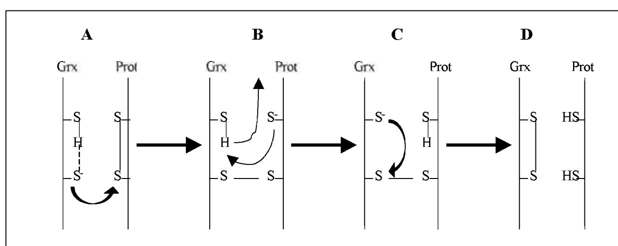


FIG. 6. **The glutaredoxin disulfide mechanism**. Reduction of disulfide bonds in target proteins by glutaredoxin.

In the monothiol mechanism concerning the reduction of protein-SG mixed disulfides, glutaredoxins utilize only the N-terminal cysteine thiol (Fig. 7) (Bushweller et al., 1992). In this reaction, glutaredoxins specifically interacts with the glutathione moiety of the glutathione mixed disulfide target and not the protein substrate, due to the glutaredoxin affinity to glutathione (Bushweller et al., 1994; Nordstrand et al., 1999). This results in the formation of a covalent Grx-SG glutathione mixed intermediate and release of the non-glutathione moiety in a reduced form. The Grx-SG mixed intermediate is reduced by a second glutathione molecule, generating oxidized glutathione (GSSG). In turn, glutathione reductase, regenerates glutathione by reducing the glutathione disulfide.

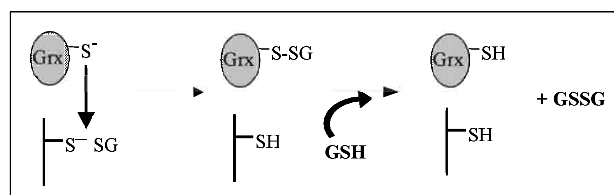


FIG. 7. **The monothiol mechanism of the glutaredoxins.** Reduction of protein glutathione mixed disulfides.

Since the reduction of protein glutathione mixed disulfides only seems to require the recognition of the glutathione moiety of the substrate, and not the substrate itself, the monothiol mechanism resulting in deglutathionylation, can thus be seen as a more general function of the glutaredoxins. Contrary to what was originally believed, glutathionylation of proteins may not only occur due to increased GSSG levels, as the formation of protein glutathione conjugates has been reported without an increase of GSSG levels (Maples et al., 1990). Protein glutathionylation is an increasingly important regulatory mechanism in biochemical processes, by reversible modification of protein thiols (Cotgreave and Gerdes, 1998). Several proteins have been detected to undergo glutathionylation due to changes in the intracellular redox environment. These include protein chaperones, cytoskeletal proteins, cell cycle regulators and enzymes of the intermediate metabolism (Lind et al., 2002). Furthermore, these types of posttranslational modification are involved in the regulation of specific transcriptional events vital to the adaptation seen in cells during oxidative stress (Klatt and Lamas, 2000). For instant, glutathionylation of Cys 62 of eukaryotic NF-κB subunit p50, and Cys 269 of c-Jun, result in loss of DNA binding activity (Klatt et al., 1999; Pineda-Molina et al., 2001). Tyrosine hydroxylase, protein kinase Cα and tyrosine phosphatase 1B are all inhibited by reversible glutathionylation (Barrett et al., 1999; Borges et al., 2002). Even though there are many reports about posttranscriptional regulation via S-glutathionylation in eukaryotic cells, only the transcription factor OxyR has so far been reported to be regulated in this manner in *E. coli* (Kim et al., 2002).

1.11 GLUTAREDOXIN IN HEALTH AND DISEASE

1.11.1 Pregnancy

High glutaredoxin activity is present in oocytes and in the ovary, and there is strong immunostaining of human Grx1 in the pinopodes of the endometrium and in cervix (Rozell et al., 1993; Sahlin et al., 2000; Stavreus-Evers et al., 2002). Due to the strong immunostaining in the endometrium it has been suggested that glutaredoxin plays an important role during implantation (Stavreus-Evers et al., 2002). Expression of the mRNA levels of glutaredoxin in the cervix are increased over 2-fold at term

pregnancy and immediately post partum. Glutaredoxin may thus be involved in the regulation of cervix ripening (Sahlin et al., 2000). There is also a significant correlation between the mRNA levels of glutaredoxin, both in normal and growth restricted pregnancies. It is believed that the glutaredoxin system is affected in placenta from pregnancies with pre-eclampsia and/or growth restriction of fetuses, and that the decrease of Grx1 levels correlates to the severity of the condition (Sahlin et al., 2000).

1.11.2 Aging

It is generally believed that aging results from oxidative damage of macromolecules from fluctuations in the balance between oxidants and antioxidants (Martin et al., 1996; Rikans and Hornbrook, 1997). There is for instance an age related decline in GSH levels that have been reported for a number of organisms. Overexpression of glutathione in transgenic *Drosophila* results in an increased life span (Sohal and Weindruch, 1996). The life span of *Drosophila* has also been increased by 30 %, by overexpression of CuZn SOD (Orr and Sohal, 1994). Mutations in signal transduction proteins have increased the lifetime of *C. elegans* and *Drosophila* (Johnson, 1990; Kenyon et al., 1993; Lin et al., 1998). Both cytoplasmic and mitochondrial superoxide dismutases (SOD) are required for the long-term survival of yeast. Overexpression of SOD1 and SOD2 increases viability in stationary phase (Longo, 1999; Longo et al., 1996). The results are consistent with studies performed with mice lacking *sod1* or *sod2* (Huang et al., 1997). Similar to eukaryotic cells, stationary phase *E. coli* become increasingly oxidized. This is despite their enhanced capacity to manage oxidative stress by the global regulator σ^S, OxyR and SoxRS (see review by (Nyström, 2002a; Nyström, 2001; Nyström, 2002b)). The oxidative stress theory on aging opens the possibility for a pivotal potential function for the thioredoxin and the glutaredoxin systems.

1.11.3 HIV/AIDS

Patients infected with the human immunodeficiency virus (HIV) are suffering from systemic oxidative stress and have an altered glutathione status in the cell (Eck et al., 1989). Several studies have shown that HIV infected patients have lower levels of both intracellular and extracellular glutathione (Buhl et al., 1989; Pace and Leaf, 1995; Staal et al., 1992). Decreased intracellular glutathione levels in HIV infected individuals have been shown for several cells including, CD4+ T cells, peripheral blood mononuclear cells and erythrocytes (De Rosa et al., 2000; Eck et al., 1989; Repetto et al., 1996). HIV infected individuals, have increased levels of protein bound glutathione, a sign of increased oxidative stress (Ghezzi et al., 2002). Moreover, N-Acetylcysteine (NAC), a prodrug of cysteine that is required for glutathione

synthesis, has been used in treatment of HIV infected patients, and was shown to improve the survival of these patients (Herzenberg et al., 1997).

The involvement of glutaredoxin in HIV pathogenesis was first reported in 1997, when HIV-1 protease was found to be a substrate for glutaredoxin (Davis et al., 1997). HIV-1 encodes an aspartyl protease, which is required for viral maturation (Darke et al., 1994). The two cysteines of the HIV-1 protease are involved in redox regulation of its activity. Modification of either of the two cysteines resulted in a decrease or loss of protease activity. Further studies showed that glutathionylation of Cys67 increased the activity several fold and also stabilized the activity *in vitro* (Davis et al., 1996). On the contrary, glutathionylation of Cys95 abolished activity. Treatment of deglutathionylated protein with glutaredoxin resulted in a 3-5 fold higher activity than the reduced form. Glutaredoxin preferentially deglutathionylates Cys95. Human glutaredoxin may also be incorporated in the HIV virion (Davis et al., 1997).

1.11.4 Neurodegenerative diseases

Dopamine neurons are sensitive to oxidative stress, and this is for instance the case in Parkinson's disease. Parkinson's disease is a neurodegenerative disorder that results in a mass destruction of the nigrostriatal dopamine system. A clear connection between dopamine turnover and glutathione oxidation has been reported (Spina and Cohen, 1989). Tyrosine hydroxylase is the initial and rate limiting enzyme in the biosynthesis of the neurotransmitter dopamine. It lacks disulfides, but the cysteine in the enzyme can be regulated by glutathionylation. As previously mentioned tyrosine hydroxylase can be reduced and thus reactivated by glutaredoxin. It has thus been suggested that the glutaredoxin system may regulate the dopamine biosynthesis under conditions of oxidative stress or drug induced toxicity (Borges et al., 2002).

There are today increasing evidence that oxidative stress is involved with the pathogenesis of Alzheimer's disease. Grx1 mRNA levels in neurons from Alzheimer's disease were reduced, suggesting a potential role of glutaredoxin in the pathogenesis of the disease (Ginsberg et al., 2000).

1.11.5 Neoplastic disease and drug resistance in cancer

Pancreatic cancer is a solid highly malignant cancer with poor prognosis. Grx1 is overexpressed in pancreatic cancer in comparison to normal parental tissue. A correlation between the increased levels of glutaredoxin and drug resistance towards the drug cis-diamminedichloroplatinum has been observed (Nakamura et al., 2000).

Glutaredoxin was 4-fold upregulated in breast tumor cells. In this case, resistance to a chemotherapeuthic drug, adriamycin, was associated with the increased levels of glutaredoxin (Wells et al., 1995).

Cis-platin is a chemotherapeutic drug that can inhibit glutaredoxin activity. The inhibition studies were performed *in vitro* under anaerobic conditions and were shown to be irreversible (Wells et al., 1991). A glutathione adduct of cis-platin also inhibits glutaredoxin activity *in vitro* in a dose dependent manner (Arner et al., 2001).

1.11.6 Atherosclerosis

As with other diseases, oxidative stress is believed to be an important factor for atherogenesis. In atherosclerotic lesions, infiltrating macrophages highly expresses glutaredoxin. Western blot analysis demonstrated that hydrogen peroxide stimulated the expression of glutaredoxin in time- and dose-dependent manners. These results suggest the possible role of glutaredoxins as antioxidants, conferring protecting against the formation of atherosclerotic lesions (Okuda et al., 2001).

1.12 PERIPLASMIC REDOX ACTIVE ENZYMES

Many proteins require disulfide bonds for their proper fold and function. Over the ten last years it has become clear that that disulfide bond formation, reduction and isomerisation are all catalysed processes (for more details se reviews by (Debarbieux and Beckwith, 1999; Fabianek et al., 2000; Raina and Missiakas, 1997; Rietsch and Beckwith, 1998; Ritz and Beckwith, 2001)). In eukaryotic cells protein disulfides are formed in the endoplasmic reticulum, while in prokaryotes like *E. coli*, the process is normally catalysed in the periplasmic space.

1.12.1 DsbA-DsbB

The periplasmic space contains two enzymes that promote the formation of disulfides, the thiol-disulfide oxidoreductase DsbA and a cytoplasmic membrane protein DsbB (Bardwell et al., 1991). DsbA is a small (21 kDa) monomeric protein belonging to the thioredoxin superfamily, and has a “classical” active site with the CXXC motif. The three-dimensional structure showed that DsbA consists of two domains. One domain has a thioredoxin like fold, and the other is a compactly folded helical domain (Martin et al., 1993). DsbA is responsible for oxidizing thiols in newly synthesized and translocated proteins. The redox potential (E_0') of purified DsbA is -0.122 V, in agreement with the oxidizing properties of this protein (Wunderlich and Glockshuber, 1993). The low pKa value (3.5) of the N-terminal C30 is believed to contribute to the oxidizing power of DsbA (Nelson and Creighton, 1994). The reaction between the reduced substrate and the oxidized form of DsbA results in an electron transfer to DsbA. Null mutants for *dsbA* have a pleiotropic phenotype. They are more sensitive to DTT, and benzylpencillin, as well as to Cd^{2+}, Zn^{2+} and Hg^{2+} and they lack active alkaline phosphatase, β-lactamase and the outer membrane protein OmpA

(Bardwell et al., 1991; Battistoni et al., 1999; Kamitani et al., 1992; Metheringham et al., 1995; Rensing et al., 1997; Stafford et al., 1999; Yamanaka et al., 1994). Other processes that are affected are mobility (disrupted flagellar assembly), and infection sensitivity to phage M13 (defected F pilus assembly) (Bardwell et al., 1991; Dailey and Berg, 1993). Other phenotypic characteristics include the lack of holocytochrome *c*, defects in enterotoxin I secretion and folding of periplasmic Cu,Zn superoxide dismutase (SOD). Homologous proteins have been found in several proteobacteria, with sequence identities between 29 % and 97 %.

The reoxidation of DsbA is performed by DsbB. DsbB is a 20 kDa cytoplasmic membrane protein with four transmembrane segments and two periplasmic loops (Bardwell et al., 1993). Each of the loops contains a pair of essential cysteine residues (Cys41, Cys44 and Cys 104, Cys130) that form disulfide bonds *in vivo*. It is believed that the disulfide bond from Cys41 and Cys44 is intramolecularly transferred to Cys104 and Cys130 and then to the active site cysteines in DsbA. Disulfide linked heterodimers, between Cys30 of DsbA and Cys104 of DsbB have been captured, suggesting that the oxidation of DsbA is a pure protein-protein interaction with DsbB (Guilhot et al., 1995; Kishigami and Ito, 1996). Recently the redox potential of the two disulfides in DsbB were determined to –271 and –284 mV, which is considerably lower than the disulfide of DsbA. This makes DsbB unsuitable to function as an oxidant to DsbA. It is therefore suggested that oxidation of DsbA by DsbB possibly occurs via a direct quinone reduction instead of via thiol disulfide exchange as it was assumed (Inaba and Ito, 2002; Regeimbal and Bardwell, 2002). The reoxidation of DsbB is further dependent on the presence of either cytochrome *bd* or *bo* and of either a menaquinone or ubiquinone electron acceptor. Cytochrome *bo* is the preferred electron acceptor during aerobic growth, while cytochrome *bd* is used under more anaerobic conditions (Bader et al., 2000). Mutants for DsbB are deficient in formation of disulfides in periplasmic proteins and are sensitive to DTT and benzylpenicillin (Missiakas et al., 1993). Cytochrome *c* maturation is also inhibited in *dsbB* null mutants (Metheringham et al., 1996; Sambongi and Ferguson, 1996).

1.12.2 DsbC-DcbD

In the *E. coli* periplasm, the isomerizations of non-native disulfide bonds are carried out via similar pathway as DsbA-DsbB. This pathway consists of the disulfide bond isomerase DsbC and the cytoplasmic membrane protein DsbD. DsbC is a soluble periplasmic protein that forms a homodimer composed of two monomers of 216 amino acids (23.5 kDa), each containing four cysteine residues (Missiakas et al., 1994). Null mutants for *dsbC* have defects in the disulfide bond formation of periplasmic proteins (but not as much as in the *dsbA* and *dsbB* mutants), and lose their ability to correctly fold periplasmic proteins with multiple disulfides (Joly and

Swartz, 1997; Rietsch et al., 1996). DsbC exhibits two distinct functions, the isomerase/reductase activity, which is located at the N-terminus of the protein and the chaperone activity, which is contributed by the C-terminus. The chaperone activity of DsbC has been shown to assist the refolding of misfolded proteins *in vitro* (Darby et al., 1998). The N-terminus of DsbC is responsible also for its dimerisation property. A mutant resulting in a monomeric protein exhibited no chaperone activity. The monomeric DsbC mutant can complement null mutants for *dsbA*, suggesting that DsbC is turned from a disulfide isomerase into an oxidase (Bader et al., 2001). This can be compared with the eukaryotic protein disulfide isomerases, which is dimeric and contain two active sites.

DsbD is a cytoplasmic membrane protein and it is responsible for the reduction of DsbC. DsbD is a 565 amino acid polypeptide, composed of a central hydrophobic domain (β) containing eight potential transmembrane segments and two periplasmic segments, one thioredoxin-like domain at the C terminus (γ) and one at the N terminus (α). DsbD contains a number of essential cysteine residues that are conserved among the bacterial species and were shown to be essential for the DsbD function (Stewart et al., 1999). Reducing equivalents are initially transferred from NADPH to TrxR, thioredoxin, DsbD and finally to DsbC. The electrons in DsbD are transferred to the hydrophobic β domain, to the thioredoxin like γ domain, to the α domain, and finally to DsbC where they reduce the active site cysteines (Collet et al., 2002). Lack of DsbD leads to hypersensitivity to DTT and benzylpenicillin, and double mutants for *dsbCdsbD* are not as motile as their parental strain (Missiakas et al., 1995).

1.12.3 CcmG, CcmH, DsbG

E. coli contains three more thiol redox periplasmic proteins, CcmG, CcmH and DsbG. CcmG and CcmH are both involved in the complex pathway of cytochrome c biogenesis (Thony-Meyer, 2002). CcmG is a 20 kDa protein with an N-terminal membrane anchor and faces the periplasm with its hydrophilic C-terminal domain containing the active site (Fabianek et al., 1998). CcmG is reduced by DsbD, in the same manner as the one described above for DsbC. The CcmG family shares a conserved sequence GVXGXPE at the C-terminus that may specify protein-protein interactions (Fabianek et al., 1997). The crystal structure of CcmG revealed a thioredoxin fold with an unusually, acid active site, and a groove formed from two inserts in the fold (Edeling et al., 2002). Point mutations of one or both cysteine residues of the active site in the chromosomal *ccmG* gene produce strongly decreased levels of holocytochrome *c* (Fabianek et al., 1998). In contrast to many other oxidoreductases, CcmG has high substrate specificity, since it did not show thiol disulfide reductase activity in the classical insulin assay, nor did the *ccmG* mutation

affect the refolding or activity of the periplasmic alkaline phosphatase (Monika et al., 1997; Page and Ferguson, 1997).

CcmH is a membrane bound protein with the conserved motif LRCXXC exposed into the periplasm (Fabianek et al., 1999). CcmH has a reducing function during cytochrome *c* maturation (Monika et al., 1997). During anaerobic growth, when *E. coli* normally synthesizes *c*-type cytochromes, only the second cysteine residue of the CXXC active site of CcmH was found to be essential for cytochrome *c* maturation. In contrast, when cells are grown under aerobic conditions, both cysteines are required (Fabianek et al., 1999).

DsbG is synthesized as a 27.5 kDa precursor protein and is processed in the periplasm to a mature protein of 25.7 kDa. DsbG share sequence homology with DsbC (29 %), and has a similar redox potential (-125 mV) (Andersen et al., 1997; Bessette et al., 1999). Like DsbC, DsbG forms a homodimer with a redox reactive CXXC motif, which is reduced by DsbD (Bessette et al., 1999). The precise function of DcbG is not known, but the *dsbG* null mutant results in accumulated reduced periplasmic proteins. Furthermore, the mutant cells were not viable unless *dsbA* or *dsbB* were overexpressed or oxidizing compounds were added to the medium (Bessette et al., 1999).

TABLE VI

Summary of the periplasmic redox active enzymes

Gene	Protein	MW of monomer (kDa)	Localization	Biological function	Sensitivity	Supressors
dsbA	DsbA	21	Periplasm	Thiol oxidant	DTT, Benzylpencillin, Cd^{2+}, Zn^{2+}, Hg^{2+}	DsbD mutations, High levels DsbC
dsbB	DsbB	20	Cytoplasmic membrane	Oxidant	DTT, Benzylpencillin	Oxidant (GSSG, cysteine) DsbD mutation
dsbC	DsbC	23	Periplasm	Disulfide bond Isomerase, Chaperone	DTT	Reductant (low levels of DTT)
dsbD	DsbD	50	Cytoplasmic membrane	Reductant	DTT, Copper	Reductant (low levels of DTT or GSH)
dsbE/ ccmG	DsbE/Ccm	20	Periplasm	Cytochrome c Biogenesis		
ccmH	CcmH		Periplasm	Cytochrome c Biogenesis		
dsbG	DsbG	26	Periplasm	Disulfide bond isomerase, Chaperone		

1.13 OXIDATIVE STRESS AND ANTIOXIDANT SYSTEMS

Oxidative stress occurs when cells are exposed to elevated levels of reactive oxygen species, such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and alkyl hydroperoxide ($^{\bullet}OH$). Oxidative stress can lead to DNA damage, thus mutations, as well as lipid peroxidation, disassembly of iron-sulfur clusters, disulfide bond formation, protein carbonylation etc. In order to protect cells against the damage caused by oxidative stress, cells produce a number of antioxidant enzymes. Both thioredoxins and glutaredoxins have been shown to have a protective role against oxidative stress (see reviews by (Carmel-Harel and Storz, 2000; Holmgren, 2000; Ritz and Beckwith, 2001)). Apart from the protective role against oxidative stress of the glutaredoxins in *E. coli*, glutaredoxins from several other organisms have shown similar effects. For instance, all yeast glutaredoxins participate in the protection against oxidative stress (Grant, 2001; Herrero and Ros, 2002). The expression of both dithiol yeast glutaredoxins (Grx1 and Grx2) are induced in response to various stress conditions including oxidative, osmotic, and heat stress (Grant et al., 2000). The expression of both genes is regulated at the transcriptional level, via the stress-responsive STRE elements (Grant et al., 2000). Yeast Grx1 null mutants are sensitive to oxidative stress induced by superoxide anion, whereas a strain lacking Grx2 or the monothiol glutaredoxin Grx5 are sensitive to hydrogen peroxide (Luikenhuis et al., 1998; Rodriguez-Manzaneque et al., 1999). The expression of mammalian glutaredoxin is for instance induced after exposing cells to TPA or ultraviolet B radiation (UVB), both treatments known to induce oxidative stress (Kumar and Holmgren, 1999; Rosen et al., 1995).

1.13.1 Transcription factors affecting antioxidant defenses

Two transcription factors that clearly protect against oxidative stress are OxyR and SoxRS. The transcription factor OxyR regulates the response to hydrogen peroxide and the transcription factors SoxR and SoxS regulate the adaptive response to superoxide generating compounds (reviewed by (Pomposiello and Demple, 2001; Storz and Imlay, 1999)).

1.13.1.1 OxyR

OxyR is a transcription factor that activates the expression of several antioxidant defensive genes in response to elevated levels of hydrogen peroxide. The response after hydrogen peroxide treatment is remarkably rapid, with the highest gene expression seen between one and five minutes (Michan et al., 1999). Genes affected are for instance *katE* (hydroperoxidase), *ahpCF* (alkylhydroperoxide reductase), *gor*

(glutathione reductase), *grxA* (glutaredoxin 1), *trxC* (thioredoxin 2) among others (Christman et al., 1985; Ritz et al., 2000; Tao, 1997; Zheng et al., 2001). The OxyR protein consists of 305 amino acids and has a mass of 34 kDa (Christman et al., 1989). The N-terminus contains a helix-loop helix DNA binding motif and is connected to the C-terminus by a flexible linker that is sensitive to proteolytic digestion (Choi et al., 2001). The levels of OxyR do not change in cells treated with hydrogen peroxide, but the regulation is rather post-translational (Storz et al., 1990). OxyR normally exist in an inactive reduced form that can be oxidized by hydrogen peroxide to the active, DNA-binding form. Oxidation of Cys198 and Cys208 to intracellular disulfide gives active OxyR. Cellular exposure to 100-1000 μM hydrogen peroxide results in the oxidation of these cysteines to form intermolecular disulfide bond. The *in vivo* oxidation reaction is fast and is completed within 30 seconds. Only the oxidized form can bind DNA at the promoter site, to stimulate transcription by protein-protein interaction with RNA polymerase. Footprinting studies showed that OxyR binds to the promoters as a tetramer (Toledano et al., 1994). The crystal structure revealed that the disulfide bond formation between the redox sensitive cysteines leads to a large structural change within the regulatory domain (Choi et al., 2001). Grx1 and Trx1 are able to reduce, and thus deactivate OxyR *in vitro*, but Grx1 seems to be the preferred reductant *in vivo* (Åslund et al., 1999; Zheng et al., 1998). Since Grx1 itself is regulated by OxyR, the response to hydrogen peroxide is autoregulated.

A mutant *E. coli* strain overexpressing continuously OxyR, overexpresses the OxyR regulated genes, and is more resistant to hydrogen peroxide than its wild type parental strain. In accordance, null mutants for *oxyR* are more hypersensitive to hydrogen peroxide and fail to activate the OxyR regulated genes. In a wild type strain, the OxyR protein is constitutively produced, but under the control of the cAMP-CRP complex, transcription of the *oxyR* gene increases during exponential growth and decreases upon transition to stationary phase. In null mutants for *cya* or *crp,* no increase in the expression of OxyR can be observed. On the other hand, a null mutant for *rpoS* allowed OxyR expression to increase as the cells entered stationary phase (Gonzalez-Flecha and Demple, 1997). Recent studies have shown that the OxyR protein can exist in a S-nitrosylated (S-NO), S-glutathionylated (S-SG), and hydroxylated (S-OH) state *in vivo*. The post-translational modification of the proteins regulatory thiol (Cys199) is transcriptionally active, but differs in structure, cooperative properties, DNA binding affinity, and promoter activity, with glutathionylated OxyR having the highest transcriptional activity (Kim et al., 2002).

1.13.1.2 SoxRS

Inducible resistance to superoxide generating agents is dependent on the integrity of the *soxRS* locus, which encodes two separate transcription activators, the SoxR and SoxS proteins (Greenberg et al., 1990; Tsaneva and Weiss, 1990). SoxR is a homodimer of 17 kDa subunits, with each monomer containing a [2Fe-2S] cluster, and it belong to the MerR family of transcription factors (Amabile-Cuevas and Demple, 1991; Hidalgo et al., 1995; Wu et al., 1995; Wu and Weiss, 1991). The SoxR protein is constitutively expressed at low levels and is activated upon exposure to superoxide generating agents or nitric oxide (Pomposiello and Demple, 2001). The SoxR activity is controlled by the oxidation state of the [2Fe-2S] cluster, which undergoes a one-electron oxidation and reduction (Hidalgo et al., 1995; Wu et al., 1995). In the reduced state, SoxR binds to the DNA without activating soxS transcription. Once it is oxidized, the transcriptional activity is activated without affecting the DNA binding affinity (Ding et al., 1996; Gaudu and Weiss, 1996). Furthermore, it has been shown that the metal centers of SoxR are not required for the initial folding of SoxR, were mutation of any of the cysteine residues, resulting in lack of [2Fe-2S] clusters, yields a stable dimeric protein that tightly binds to DNA (Bradley et al., 1997; Hidalgo and Demple, 1994). Oxidized SoxR is rapidly reduced once the oxidative stress is removed (Ding and Demple, 1997). The regulation of the soxRS regulon occurs in two steps (Nunoshiba et al., 1992; Wu and Weiss, 1992). Under conditions of oxidative stress, SoxR is activated by oxidation or nitrosylation by nitric oxide, which then can activate the transcription of the *soxS* gene.

SoxS is a protein of 13 kDa that may activate the expression of at least 17 genes or operones, resulting in increased resistance to oxidants, antibiotics, organic solvents and macrophage-generated nitric oxide. Genes upregulated by SoxS include manganese superoxide dismutase (*sodA*), the DNA repair enzyme endonuclease IV (*nfo*), aconitase A (*acnA*) and fumerase C (*fumC*) (Reviewed by (Storz and Imlay, 1999)). The proteins regulated by the soxRS system results in a mechanism to avoid oxidative damage which includes, scavenging of oxidants, DNA repair, reduced permeability and excretion of toxicants (Greenberg et al., 1990).

Null mutants for *gshA*, *trxA* or *trxB* do not affect the induction of a *soxS-lacZ* fusion in response to paraquat. On the other hand, the *soxS-lacZ* induction was significantly reduced in a double mutant for *gshAtrxA*, indicating that the glutaredoxin or the thioredoxin system is required for the activity of the SoxR protein (Ding and Demple, 1996; Ding and Demple, 1998).

1.13.2 Additional proteins belonging to the thioredoxin structural superfamily with involvement in oxidative stress

Apart from the thioredoxin and the glutaredoxin systems described above, there are other enzymes with Trx/Grx fold whose antioxidant activity is dependent on GSH or TrxR. Glutathione transferases, glutathione peroxidases and peroxiredoxins are some of these enzymes.

1.13.2.1 Glutathione transferase

Glutathione S-transferases (GSTs) play important role in higher eukaryotes in the binding, transformation and detoxification of a wide variety of both endogenous and exogenous compounds, such as carcinogenic, mutagenic, toxic and pharmacologically active substances (Chasseaud, 1979). GST enzymes have been studied extensively in eukaryotes since their discovery in 1961. GSTs are usually active as dimers, and exists as both homodimeric and heterodimeric proteins. They can be expressed constitutively or by induction of a variety of both natural and xenobiotic compounds (reviewed by (Vuilleumier, 1997)).

GSTs have been defined to different classes, α, μ, π, σ, ζ, ω in mammals, ϕ and τ in plants δ in insects and β in bacteria (Sheehan et al., 2001). *E. coli* has one GST enzyme that has been cloned and characterized, and shows high affinity to GSH (K_m of 40 μM) (Iizuka et al., 1989; Nishida et al., 1994). *E. coli* GST was isolated as a dimer of an identical subunit of 23 kDa, each consisting of 201 amino acid residues. The *E. coli* GST conserves overall constructions common to the eukaryotic enzymes, in polypeptide fold, dimeric assembly, and glutathione binding site (Nishida et al., 1998). Its amino acid sequence is 54 % identical to that of *Proteus mirabilis* GST, but less than 20 % any of the eukaryotic GSTs.

At a functional level, GST can be distinguished in two types. The first, most common reacts with electrophilic compounds to yield stable glutathione conjugates. The second type, yields metabolites and energy for the bacterial growth. The latter enzymes may be quite specific for bacteria, since GSTs from eukaryotes appear not to be active in central metabolism, but rather to specialize in detoxification reactions. In bacteria, the few known glutathione *S*-transferases (e.g. dichloromethane dehalogenase, 1,2-dichloroepoxyethane epoxidase and tetrachlorohydroquinone reductase), are catabolic enzymes with an essential role for growth on recalcitrant chemicals (e.g. dichlormethane). Glutathione conjugates, produced by bacterial electrophilic compounds, might be disposed through excretion. *E. coli*, possesses two glutathione-gated potassium channels, KefB and KefC, which are activated by glutathione-S-conjugates formed with methylglyoxal. Activation of these channels leads to

cytoplasmic acidification, which protects the cells during electrophilic attack (Ferguson et al., 1995).

1.13.2.2 Glutathione peroxidase

Glutathione peroxidases are believed to be one of the most important defenses against peroxides in mammalian cells. Bacteria lack such activity, but it has been detected in *S. cerevisiae* were three genes have been identified (GPX1, GPX2, GPX3) (Galiazzo et al., 1987). Null mutants for the gene encoding for GPX3, are hypersensitive to hydrogen peroxide and *t*-butyl hydroperoxide (Inoue et al., 1999).

1.13.2.3 Peroxiredoxins

Peroxiredoxins (Prxs) form a large family of antioxidant enzymes that is divided into several molecular clades and is spread over all living domains. They are low efficiency peroxidases using thiols as reductants, and differ from the other peroxidases in that they do not contain metals ions or prosthetic groups. This family of proteins was discovered as late as in 1988, when a thiol-specific antioxidant protein (TSA) was identified (Kim et al., 1988). It was not until 1994 that the antioxidant efficacy of TSA could be attributed to peroxidase activity, with the specific donor substrate being thioredoxin (Chae et al., 1994). All TSA related proteins are today collectively called 'peroxiredoxins' (Chae et al., 1994). The Prxs superfamily can be divided into two subgroups, the 1-Cys and the 2-Cys peroxidases, in accordance to the presence of one or two conserved cysteine residues in the catalytic mechanism of the enzyme.

1.13.2.3.1 Thioredoxin peroxidases

In *E. coli* there are two enzymes that have peroxidase activity that require thioredoxin and thioredoxin reductase for their catalytic cycle. The first enzyme is a 20 kDa periplasmic protein (encoded by *tpx*) (Cha et al., 1995). It was discovered as a peroxidase, protecting cells from DNA damage and glutamine synthetase inactivation that was caused by metal-catalyzed oxidation. In *E. coli* substitution of serine for Cys94 results in complete loss of Prx activity. Null mutants for *tpx* are hypersensitive to the superoxide generating compound paraquat and slightly sensitive to hydrogenperoxide and *t*-butyl hydroperoxide (Cha et al., 1996).

The second enzyme is a 18 kDa protein called bacterioferritin-comigratory protein (encoded by *bcp*) and belongs to the 1-Cys peroxidases (Jeong et al., 2000). It shows homology to alkylhydroperoxide reductase and has been found to have similar antioxidant activity as the *tpx*-encoded protein. Bcp mutants are hypersensitive to hydrogenperoxide and *t*-butyl hydroperoxide.

1.13.2.3.2 Alkylhydroperoxide reductases

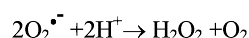
Alkylhydroperoxide reductase (encoded by *ahpCF*) is composed of two components, a 22 kDa AhpC subunit that acts as a substrate and a 52 kDa AhpF flavoprotein that uses NADH or NADPH to reduce oxidized AhpC. Alkylhydroperoxide reductase converts lipid hydroperoxides and other ROOH to the corresponding alcohols. The enzyme was discovered in an OxyR constitutive active strain that was more resistant to cumene hydroperoxide than the parental wild type strain (Jacobson et al., 1989). OxyR regulates the expression of *ahpCF* (Tartaglia et al., 1989). Null mutants for *ahpCF* are slightly more sensitive to hydrogen peroxide and highly more sensitive to cumene hydroperoxide (Storz et al., 1989).

An *E. coli* strain that lacks thioredoxin reductase and glutathione reductase grows extremely poorly under aerobic conditions unless a reducing agent such as dithiothreitol (DTT) is present (Bessette et al., 1999). This strain can be rescued by AhpC. It is a mutation occurring at high frequencies in the gene *ahpC* that leads to restored cell growth in this strain. This mutation, leads to the addition of one amino acid, which in turn converts the AhpC protein from a peroxidase to a disulfide reductase, using Grx1 as substrate (Ritz et al., 2001).

1.13.3 **Other antioxidant defence systems in *E. coli***

1.13.3.1 Superoxide dismutase (SOD)

E. coli contains the manganese and iron containing cytosolic SODs (MnSOD and FeSOD respectively), and a periplasmic copper-zink containing SOD (Cu/ZnSOD). In 1986 an *E. coli* mutant was constructed lacking both the iron and manganese containing SOD isozymes. The null mutant exhibited growth defects, but only when oxygen was present. This phenotype was also only apparent when both enzymes were absent (Carlioz and Touati, 1986). SOD catalyses the reaction were superoxide is conjugated to form hydrogen peroxide and oxygen:

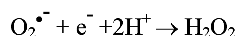


$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

1.13.3.2 Superoxide reductase (SOR)

Recently a novel type of superoxide scavenging enzyme, was discovered, catalyzing the direct reduction of superoxide. These iron containing proteins were named 'Superoxide reductases' (ROS), and have now been well characterized in several organisms.

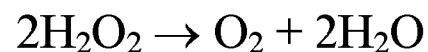
In 1996 attempts were made to clone a SOD gene from *Desulfoarculus baasii*. Surprisingly the isolated gene was a desulfoferrodoxin (Dfx), a protein that had been previously described but whose function was unknown (Pianzzola et al., 1996). Dfx was assisting in the scavenging of superoxide, even though the SOD activity of Dfx was modest (Liochev and Fridovich, 1997). Two independent studies confirmed that Dfx and its homologues enzymes may acts as superoxide reductases. The superoxide reductase activity was confirmed by purifying and characterizing the enzyme in *Pyrococcus furiosus*, and in *Desulfoarculus baasii* (Jenney et al., 1999; Lombard et al., 2000). In *Pyrococcus furiosus* superoxide reductase uses electrons from NAD(P)H and NAD(P)H-rubredoin oxidoreductase, to reduce superoxide to hydrogen peroxide, which in turn is reduced to water by peroxiredoxins.



$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$$

1.13.3.3 Catalases

Catalases are found in most aerobic organisms and employ a two-electron transfer mechanism in the dismutation of hydrogen peroxide to water and oxygen.



$$2H_2O_2 \rightarrow O_2 + 2H_2O$$

Two catalases (or hydroperoxidases) with very different properties have been identified in *E. coli*, hydroperoxidase I (HPI), and hydroperoxidase II (HPII). HPI was first described as a bifunctional enzyme exhibiting catalatic and *o*-dianisidine peroxidatic activity (Claiborne and Fridovich, 1979a). After a catalase deficient mutant had ben isolated, the gene encoding for HPI, *katG*, was identified, subcloned and sequenced (Loewen et al., 1985; Loewen et al., 1983; Triggs-Raine et al., 1988; Triggs-Raine and Loewen, 1987). HPI has a tetrameric structure of identical 80 kDa subunits and it contains two molecules of protoheme IX (Claiborne and Fridovich, 1979b). It is induced in response to hydrogen peroxide and it is part of the *oxyR* regulon (Morgan et al., 1986).

HPII was characterized as a monofunctional catalase, containing a modified heme that gave the enzyme its characteristic green color (Claiborne et al., 1979; Loewen and Switala, 1986). HPII is a tetramer of identical 84 kDa subunits, encoded by *katE*. In contrast to HPI, HPII synthesis does not respond to hydrogen peroxide and is not regulated by OxyR. HPII remains low throughout early exponential phase, and increases six to nine folds in the transient to stationary phase (Loewen et al., 1985). The induction requires σ^s (Mulvey et al., 1990). HPII is also important for long-term survival under starvation conditions (Mulvey et al., 1990).

1.14 RESPONSE TO CHALLENGES OTHER THAN OXIDATIVE STRESS

Organisms have acquired numerous responses required for their survival during environmental variations. They have the ability to sense and adapt to changes in temperature, osmolarity, nutrients and hydrogen ion concentration (pH). Part of the bacterial stress responses and their connection to the thioredoxin and glutaredoxin systems are briefly reviewed.

1.14.1 The stringent response

During nutrient starvation, cells of *E. coli* elicit stringent control to conserve energy, the phenomenon termed "stringent response". This control encompasses a rapid reduction in ribosomal RNA biosynthesis during cellular starvation (Cashel et al., 1996). The adaptation to nutrient stress is characterized by downregulation of nucleic acids and protein synthesis and the simultaneous upregulation of protein degradation and amino acid synthesis. The hallmark of the stringent control is the accumulation of guanosine-3',5'-tetraphosphate (ppGpp), which by binding to RNA polymerase (RNAP) (Chatterji et al., 1998), causes a rapid reduction in ribosomal RNA transcription, probably by reducing the stability of the open promoter/RNAP complexes at ribosomal RNA promoters (Bartlett et al., 1998; Gourse et al., 1998). tRNA was found to be regulated in a similar manner, were free tRNA accumulates at the A-site of the 50s ribosome, leading to a stall in protein synthesis, in turn resulting in a reaction were ribosome bound RelA is activated to synthesise ppGpp (Haseltine and Block, 1973).

ppGpp affects the growth rate of bacteria, since the synthesis of stable RNA correlates directly with the growth rate of an organism. A steady–state level of ppGpp at exponential phase is maintained in the cytosol by the two enzymes, RelA and SpoT. RelA is a synthase, whereas SpoT primarily is a hydrolase, with synthase activity under certain conditions. A *spoTrelA* null mutant is therefore devoid of ppGpp and is polyauxotrophic (Xiao et al., 1991). The alarmone ppGpp can also act as a positive effector of gene expression, and a large number of σ^{70}-dependent genes require this nucleotide for their induction during stationary phase and starvation (Kvint et al., 2000). For instance *E. coli* Trx1 transcription is positively regulated by ppGpp (Lim et al., 2000). ppGpp is also involved in protein degradation and DNA replication (Cashel et al., 1996; Kuroda et al., 1997; Kuroda et al., 1999). The DNA replication is inhibited by ppGpp in cells during stringent response to cope with decreased cellular growth rate. It was further shown that termination of DNA synthesis, during stringency, essentially requires ppGpp and the replication protein, RTP, which is required for normal termination (Autret et al., 1999). ppGpp participates also in the DNA repair pathway in the cell (McGlynn and Lloyd, 2000).

1.14.2 Regulation of gene expression of stationary phase

Bacteria respond to different stresses with the synthesis or activation of sigma factors, which in turn regulate the transcription of genes involved in cellular responses. *E. coli* has six different sigma factors that have evolved to respond to different stresses, including starvation, heat shock, nitrogen depletion, extracytoplasmic stress, citrate-dependent iron transfer and the need for flagellin.

The transcription factor σ^s, encoded by *rpoS* was initially characterized as a regulatory protein controlling the expression of proteins involved in the starvation or stationary phase response. *rpoS* encodes a 342 amino acid, 38 kDa protein that functions as an alternative sigma factor (sigma-38) for RNA polymerase. The *rpoS* gene was first known as *katF* because of its regulatory effects on *katE*, which encodes a stationary phase specific catalase (catalase II) (Mulvey and Loewen, 1989). The gene is very similar to the previous sigma factors in *E. coli*, and due to its crucial role under stress conditions, Lange and Henge-Aronis renamed the *katF* gene to *rpoS* and designated its product σ^s (Lange and Hengge-Aronis, 1991). σ^s has been shown to control the expression of over 50 genes during the transient from exponential phase to stationary phase. Some of the gene products are involved in protection against oxidants (e.g. catalases) and repair of oxidative damage (e.g. exonuclease III). *E. coli* glutathione reductase is one protein positively regulated by σ^s in the stationary phase of growth (Becker-Hapak and Eisenstark, 1995).

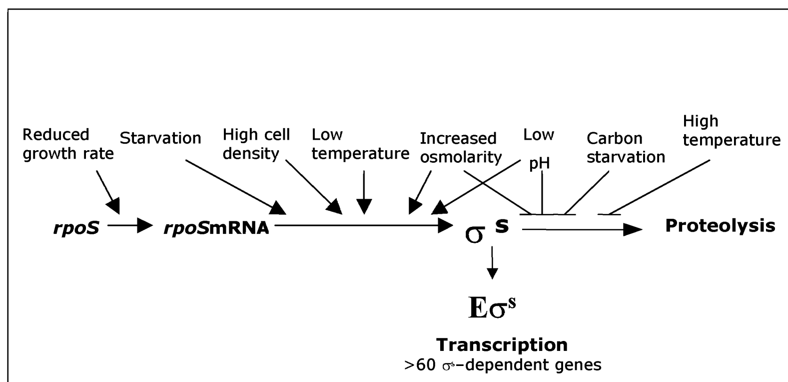


FIG. 8. **Regulation of σ^s is affected at different levels and by various stress conditions.** An increase in the cellular σ^s level can be obtained either by stimulating σ^s synthesis at the level of transcription or translation or by inhibiting proteolysis of σ^s.

σ^s is involved in many phenomena specific for stationary phase, starvation, osmotic shock, acid shock, heat shock and cold shock (Fig. 8). Protein levels of σ^s

undetectable during exponential phase, but during entry into stationary phase they are drastically increased (Jishage and Ishihama, 1995). The concentration of σ^s is controlled at the level of transcription, translation and protein stability (Lange and Hengge-Aronis, 1994). Control of *rpoS* transcription involves ppGpp as a positive regulators and the cAMP receptor protein (cAMP-CRP) as a negative one. The translation of σ^s is controlled by a number of interacting factors (HU, Hfq, DsrA RNA, OxyS, DksA and ppGpp) and the protein is sensitive to proteolysis by ClpP (Fig. 9). In mutants lacking the protease ClpP, σ^s levels at the exponential phase were similar to those in the stationary phase of wild type cells. On the other hand, ClpP levels remain the same at all stages of growth, leading to the proposition that σ^s becomes more resistant to this protease in the stationary phase (Schweder et al., 1996; Webb et al., 1999; Zgurskaya et al., 1997). The null mutant for *rpoS* has a striking phenotype where a rapid cell death follows soon after entry in the stationary phase (Eisenstark et al., 1995).

RpoS is believed to be involved in pathogenesis (Prince et al., 1994). In the enterobacticterial pathogens, *Salmonella* and *Shigella*, the wild type strain was found to be substantially more lethal to mice than the *rpoS* mutant (Lee et al., 1995; Small et al., 1994). There is also evidence that RpoS regulates genes that directly initiate cellular infectivity (Heiskanen et al., 1994; Kowarz et al., 1994). Mouse inoculation studies with *Salmonella rpoS* mutants of both wild type and virulence plasmid-cured strains suggest that *rpoS* contributes to Salmonella virulence via the regulation of chromosomal genes (e.g. the Salmonella plasmid virulence (spv) genes).

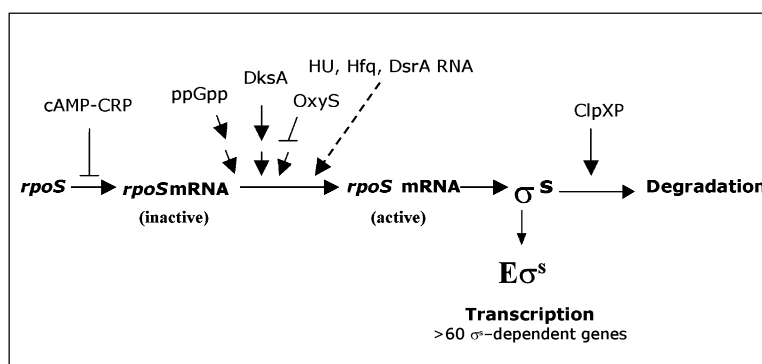


FIG. 9. **The *rpoS* translational network**. RpoS mRNA is thought to exist in at least two different conformations, one being a more closed structure (inactive), and the other being a more open and translationally competent structure (active). The translation stimulating factors HU, Hfq and DsrA RNA can bind to the rpoS mRNA and drive it to the active form. OxyS, DksA and ppGpp are likely to act more indirectly.

1.14.3 Acid stress

E. coli possesses an amazing ability to adapt and survive under acid stress. One critical host defense system that *E. coli* must overcome is the acid stress barrier present in the stomach. The mean stomach pH under fasting conditions is approximately 2.0. These levels caused by inorganic acids (H+) in the stomach can be life threatening to *E. coli*. It has evolved both constitutive and inducible defense systems to survive under these conditions. Three major functions of the cell may be affected by acid conditions, the capacity for nutrient acquisition and energy generation, cytoplasmic pH homeostasis and preservation of protein and DNA structure. It is for instance known that several types of *E. coli* DNA repair mutants are more rapidly killed under acidic conditions than their parental strain (Sinha, 1986). Low pH is also an important signal that *E. coli* has entered a potential host environment. This signal triggers the induction of many virulence genes (Lucas and Lee, 2000). Among the many proteins induced by acid stress is *E. coli* Grx2 (Arnold et al., 2001).

Three distinct, but overlapping, systems to resist acid stress have been identified in *E. coli* (Hersh et al., 1996; Lin et al., 1995; Lin et al., 1996). Which system is induced and functional, depends on the media and on the growth conditions. The first system is an oxidative or glucose-repressed acid resistance, highly dependent upon the transcription factor σ^s and repressed by glucose. In many situations this system is also dependent on the cAMP receptor protein (Castanie-Cornet et al., 1999). *S. typhimurium* mutants defective in *rpoS* or producing low levels of σ^s, are extremely sensitive to acid treatment (Audia et al., 2001). The HdeA protein, encoded by the *hdeAB* operon, is under control of σ^s and is one of the most abundant proteins in the periplasm at the stationary phase of growth (Waterman and Small, 1996). A null mutant for HdeA was found to exhibit 10 000-fold lowering in its survival compared to the parental strain after incubation at pH 3. The current belief is that HdeA and HdeB form heterodimers in the periplasm under normal conditions that dissociate at acidic pH to bind to unfolded periplasmic proteins, preventing their aggregation (Gajiwala and Burley, 2000).

The second system provides the highest level of acid resistance, allowing cells to survive at very low pH (pH of 2). This system is glutamate dependent and requires the two glutamate decarboxylase isoenzymes encoded by *gadA* and *gadB* and the γ-aminobutyric acid (GABA) antiporter GadC. At pH 2.5 only one of the *gad* genes is required for rescuing the cell, while during more acidic conditions with pH 2.0 both the genes are required. The regulation of this system is very complex, with the control occurring at the transcriptional level within a 20 bp conserved region located 50 bp from the transcriptional start of both operons (Castanie-Cornet and

Foster, 2001). It is believed that CRP normally represses the *gad* genes. When CRP-cAMP levels are high, σ^s is required for expression of *gad*. If CRP is absent or when cAMP levels are low, σ^s is not required. In the latter case, the housekeeping σ^{70} efficiently transcribes the *gad* genes. However, acid induction is still required (Audia et al., 2001). Another protein functioning as an activator of *gadA* is GadX. GadX increases the production of the glutamate decarboxylases and activates the transcription of the *gadA* and *gadB* promoters. It is proposed that GadX is a transcriptional regulator of genes required for acid resistance and virulence of enteropathogenic *E. coli* (Shin et al., 2001).

The third and last system to resist acid stress is an arginine-dependent acid resistant system. This system shows a more modest protection of the cell compare with the glutamate dependent system. This system requires arginine decarboxylase encoded by *adiA*. Arginine decarboxylase converts arginine to agmatine, resulting in the consumption of a proton and thus elevation of the cytoplasmic pH.

1.14.4 Osmosis

Bacteria can survive dramatic changes in their extracellular osmolality. Responses to osmolality changes are active or passive. Bacteria respond to osmotic upshifts in three overlapping phases. Phase one (within 1-2 min) is characterized by dehydration (loss of some cell water), phase two (after 20-60 min) by adjustment of cytoplasmic solvent composition and rehydration and finally phase three (after 1 h) by cellular remodeling (DNA/Protein synthesis, cell growth and division resumed). The response to osmotic downshift is not as well characterized, but is also believed to proceed in three phases. First water uptake in phase one, followed by extrusion of water and cosolvents in phase two and last in phase three by cytoplasmic cosolvent reaccumulation and cellular remodeling (for more details se (Wood, 1999)).

One general mechanism of response to osmotic conditions is via σ^s. Osmotic upshift results in an elevated cellular σ^s level, similar to that observed in stationary phase. The increase is a result of stimulation of *rpoS* translation as well as inhibition of the turnover of σ^s .σ^s in turn, can acts as a global regulator for the osmotic control of gene expression, and the regulation actually occurs in cells of the exponential phase (Hengge-Aronis et al., 1993). It has been suggested that the σ^{32}, σ^E and σ^s regulons co-operate closely in the management of hyperosmotic stress (Bianchi and Baneyx, 1999). CRP-cAMP can also function as a sensitive regulator to osmotic changes. The complex can function either as a repressor (e.g. *proP* P1 promoter) or as an activator (e.g. *lac* promotor) (Landis et al., 1999).

Mechanosensitive channels (MscS, MscM, MscL) are central in the release of cytoplasmic solutes to achieve a rapid reduction of pressure during the transient from high to low osmolarity (Berrier et al., 1992). They are located in the

cytoplasmic membrane of *E. coli*, and detect osmolality changes indirectly as changes in mechanically imposed membrane stress. The MscL is the only mechanosensitive channel cloned. The *mscL* gene encodes a 15 kDa protein with two transmembrane domains (Blount et al., 1996). Two-dimensional crystals of the channel indicated a homohexameric structure (Saint et al., 1998). A more recent investigation proposed that it rather consists of five subunits (Sukharev et al., 1999). Cleavage of the external loop of each monomer, results in a functional channel, but with dramatically increased mechanosensitivity. It was suggested that the loop acts as a spring that resists the opening of the cannel and promotes its closure when the channel is opened (Ajouz et al., 2000).

Apart from the low molecular weight compounds (ions, metabolites and osmoprotectants) released by the mechanosensitive channels, some cytoplasmic proteins are also excreted from *E. coli* upon osmotic downshock; among them are Trx1 (Lunn and Pigiet, 1982). Levels of *E. coli* Trx1 are also elevated upon osmotic upshock (Scharf et al., 1998). In a shift back to low osmolarity conditions, Trx1 is secreted via the mechanosensitive channel MscL (Ajouz et al., 1998). It could be that the high levels of Trx1 may be deleterious after osmotic upshock, or it could be that Trx1 may be needed in the periplasm. Trx1 remains trapped in the periplasm, unless the outer membrane is disrupted by Tris-EDTA treatment (Berrier et al., 2000).

2 RESULTS AND DISCUSSION

The results on which this thesis is based on are thoroughly presented and discussed in papers (I-V). Comments and brief summary for each paper follow below.

2.1 PAPER I

Characterization of *Escherichia coli* null mutants for glutaredoxin 2

In contrast to the classical *E. coli* Grx1 and Grx3, Grx2 is a larger protein, of 24 kDa, with has almost no homology with the other glutaredoxins of *E. coli*, except the conserved active site (CPYC). Little was known about this protein, so to improve the understanding of glutaredoxin function, a null mutant for the Grx2 gene (*grxB*) was constructed and combined with null mutants for the other glutaredoxins. Null mutants for *grxB* and all three glutaredoxin genes ($grxA^{-}grxB^{-}grxC^{-}$) were viable in rich and minimal media. Grx2 was found to contribute to 80 % of the total glutaredoxin activity measured by the β-hydroxyethyl disulfide (HED) assay.

Levels of intracellularly expressed alkaline phosphatase (AP) showed that Grx1 and Grx2 (but not Grx3) under certain conditions contributed in the reduction of cytosolic disulfides. However, the role of Grx1 as a reductant of disulfide bonds could be reversed to that of an oxidant under very oxidizing environments. This phenomenon had been described for the thioredoxins, but never before for the glutaredoxins.

Glutaredoxins contributed to the defence against hydrogen peroxide, with g*shA* and *grxB* minus cells being more sensitive to hydrogen peroxide and other oxidants as shown by increased carbonylation of intracellular proteins of the relevant mutants, particularly in the stationary phase. Grx2 and GSH are likely to constitute therefore to the major thiol system for the protection of proteins against hydrogen peroxide induced carbonylation.

Significant upregulation of catalase activity was observed in null mutants for thioredoxin 1 and the three glutaredoxins, while upregulation of glutaredoxin activity was observed in catalase deficient strains with additional defects in the thioredoxin pathway. This shows an interconnection between the glutaredoxin and catalase antioxidant defences.

An unexpected finding was that $gor^{-}grxA^{-}grxB^{-}grxC^{-}$ stains did not grow well on minimal medium plates unless supplemented with some form of reduced sulfur (SO_3^{2-}, Met or Cys). Reduction of SO_4^{2-} to SO_3^{2-} is catalyzed in *E. coli* by PAPS reductase. Trx1, Trx2 and Grx1 can reduce the disulfide of the PAPS reductase while Grx2 and Grx3 cannot. Therefore, *E. coli* should not need any glutaredoxin to reduce sulfate to sulfite, thioredoxins should be able to compensate for this fully. However,

since *gor⁻grxA⁻grxB⁻grxC⁻* cells could be rescued by monothiol Grx2, it seems that a mechanism for the activation of PAPS reductase involves a mixed disulfide with GSSG, which in turn results in an inactive enzyme. In other words, we believe that PAPS reductase is a subject to a mixed disulfide mechanism for the regulation of its activity.

2.2 PAPER II

Protein levels of *Escherichia coli* thioredoxins and glutaredoxins and their relation to null mutants, growth phase and function

The aim of this work was to further characterize the interactions and compensation of the thioredoxin and glutaredoxin systems of *E. coli*. We developed sensitive ELISAs for the two thioredoxins (Trx1, Trx2) and the three glutaredoxins (Grx1, Grx2, Grx3) of *E. coli*. We found that levels of the Grx2, Grx3 and Trx1 were highly abundant. In a wild type strain, Trx1 levels increased at the stationary phase of growth, as did the levels of Grx2. Grx3 and Trx2 levels were quite stable during growth. Grx1 levels decreased, while cells moved from the exponential to the stationary phase of growth.

The levels of the different redoxins were further analysed in different genetic backgrounds. A dramatic elevation of Grx1 (20-30-fold) was observed in null mutants for *trxAtrxC* and *trxAtrxBtrxC* while levels of the other redoxins in all combinations of examined null mutants increased about 2-3-fold. Overall, our data suggest that only Grx1 and Trx1 have strictly overlapping and specific functions, presumably the reduction of ribonucleotide reductase. The reduction of ribonucleotide reductase was examined by measurements of thymidine incorporation in newly synthesized DNA, by using different null mutant cells. This showed that it is mainly Grx1 and to a lesser extent Trx1 that are involved in the reduction of deoxyribonucleotides.

All glutaredoxin species were elevated in catalase deficient strains, particularly when combined with null mutants from the thioredoxin or glutaredoxin system, implying an antioxidant role for the glutaredoxins. However, administration of hydrogen peroxide resulted in a decrease of Grx2 (and Grx3) levels while addition of mercaptoethanol increased the amounts of both Grx2 and Grx3. Transcription of Grx2 and Grx3 is thus not likely to be regulated by OxyR.

It is known that the expression of GR is regulated at the exponential phase by OxyR (Christman et al., 1985) and at stationary phase by ppGpp (Becker-Hapak and Eisenstark, 1995). In agreement with previous results we found that GR levels were higher at the stationary phase of growth. Simultaneous elevation of GR activity and the glutaredoxin activity (*katEkatG* and *katEkatGtrxB* null mutants) was also

detected. This would fit well with the need to reduce their increased glutathione disulfide occurring as a by product of increased glutaredoxin activity in the particular strains.

2.3 PAPER III

Expression of *Escherichia coli* glutaredoxin 2 is mainly regulated by ppGpp and sigma s

We wanted to investigate the regulation of Grx2 during the transient from exponential growth to starvation. Guanosine-3',5'-tetraphoshate (ppGpp) and σ^s (RpoS), which regulate the transcription of genes in the stationary phase of growth affected dramatically the expression of Grx2. Grx2 expression was inhibited by cAMP at the exponential phase, but since it is known to function as a negative regulator for the expression of σ^S, the effect on Grx2 is more likely to be indirect through down regulation of σ^S. Grx2 levels were also positively affected by osmotic upshock. More experiments are needed to elucidate whether this is a direct effect or an indirect effect via σ^S.

OxyR, a positive effector for the expression of Grx1, did not affect the levels of Grx2 or Grx3. Grx2 levels were instead elevated in an *oxyR* null mutant. In comparison to Grx2, levels of Trx1 were mainly regulated by ppGpp but not σ^s. In accordance with the role of Grx2 as a protein of the stationary phase, null mutants for *grxB* were lysing at the stationary phase of growth and exhibited a distorted morphology.

The elevation of Grx2 levels to up to 1 per cent of total cell protein combined with the high GSH levels of the stationary phase (Loewen, 1979), and the distorted morphology of the *grxB*$^-$ cells at the stationary phase, imply a vital yet unknown function for Grx2.

2.4 PAPER IV

Redox regulation of 3′-phosphoadenylylsulfate reductase from *Escherichia coli* by glutathione and glutaredoxins

PAPS reductase is the key enzyme for the reduction of sulfate to sulfite. Prototrophic bacteria or fungi mainly use inorganic sulfate as the only supply of sulfur for the biosynthesis of amino acids and essential cofactors. In *E. coli*, Trx1 or Grx1 is essential for the reduction of sulfate. In this paper we wanted to investigate the actual reason from our previous finding that a *gor*$^-$*grxA*$^-$*grxB*$^-$*grxC*$^-$ stains did not grow well on minimal medium unless supplemented with reduced sulfur, even though thioredoxin was present (Paper I).

We found that incubation of PAPS reductase with oxidized glutathione lead to enzyme inactivation with simultaneous formation of a mixed disulfide between glutathione and the active site Cys239. Glutathionylated PAPS reductase could be reduced *in vitro* by the glutaredoxins. Furthermore, glutathionylated PAPS was observed also *in vivo* in poorly growing *gor⁻grxA⁻grxB⁻grxC⁻* expressing inactive Grx2 C9S-C12S. However, in better growing cells expressing monothiol Grx2C12S or wild type Grx2, the protein mixed disulfide species was absent. Reversible glutathionylation may thus regulate the activity of PAPS reductase (Fig 10).

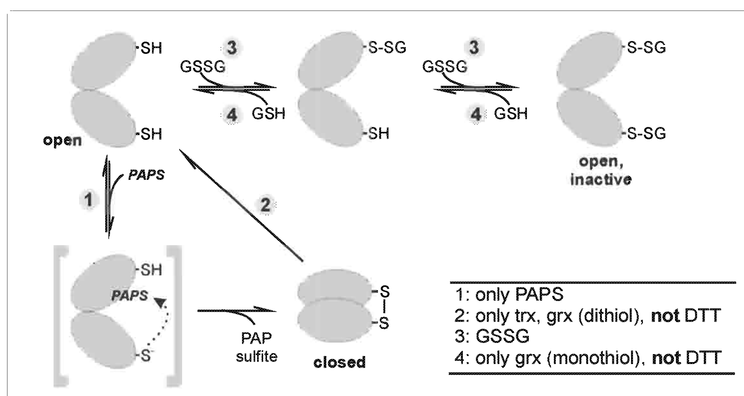


Fig. 10. **Model of the thiol-based mechanism and the conformational changes of PAPS reductase.**

Formation of protein-glutathione mixed disulfides is of physiological relevance for *E. coli,* since up to 2 % of the total glutathione content (10 - 20 μM) is in the form of protein-mixed disulfides in a wild type cell and can be even higher, as for example in *trxAgrxA* null mutants (5 - 7 %) (Miranda-Vizuete et al., 1996). In mammalian cells, extensive glutathionylation of protein substrates has been identified to include chaperons, cytoskeletal proteins, cell cycle regulators and enzymes participating in the intermediate metabolism (Lind et al., 2002). A similar study would be of great interest for the *E. coli* system.

2.5 PAPER V

Cloning and characterization of a novel *Escherichia coli* monothiol glutaredoxin

In this work we report the cloning of a novel putative monothiol *E. coli* glutaredoxin, glutaredoxin 4 (Grx4). This is a protein of 115 amino acids (13 kDa), a

monothiol active site (CGFS) and with high homology to the monothiol yeast Grx5 (37 % sequence identity). The active site of Grx4 (CGFS) is identical to that of the yeast enzymes, but in terms of size, Grx4 is close to Grx1 and Grx3 from *E. coli* of ~10 kDa. In comparison, yGrx3 is of 285 amino acids, yGrx4 of 244, yGrx5 of 151 and the human PICOT of 335. *E. coli* Grx4 with 115 amino acids is thus the smallest of the monothiol family. The predicted fold for Grx4 is suggestive of the classical alternated α/β structure for Trx/Grx. Structural analysis of Grx4 by CD showed a mixed α/β fold but also a higher helical content in comparison to Grx1.

Grx4 lacked activity in the classical HED assay that measures the reduction of the low molecular weight mixed disulfide between β-mercaptoethanol and oxidized glutathione. To examine whether the lack of activity of Grx4 in the HED assay was due to its CGFS monothiol active site, we overexpressed Grx4 mutants with a dithiol (CGFC, CPYC) in the active site. These mutants were not active in the HED assay. Since overexpressed Grx4 was folded properly (CD data), it could be that the HED-gluathione mixed disulfide is simply not a substrate for Grx4. Grx4 was active however in the reconstitution of [4Fe-4S] cluster in apoFNR to give FNR. FNR is a transcriptional factor that upregulates the transcription of proteins in conditions of low oxygen.

Grx4 was highly abundant, with levels up to 6 μg/mg of total soluble protein. Like the other three glutaredoxins of *E. coli*, Grx4 was upregulated in mutants lacking the thioredoxin system. As is the case for Grx2 and Trx1, Grx4 was upregulated at the stationary phase of growth with an almost three-fold increase. The regulation of Grx4 thus differs from that of the yeast monothiol glutaredoxins, which were, all at their maximum expression during the exponential phase of growth and their mRNA levels decreased under detection limits at the stationary phase (Rodriguez-Manzaneque et al., 1999). Levels of Grx4 were regulated at the stationary phase by ppGpp, but not RpoS. This regulation by ppGpp is similar to that of Trx1 (Lim et al., 2000).

3 CONCLUSIONS

The results in this thesis expanded the understanding of the role of the glutaredoxin system in *E. coli*. More specifically:

3.1.1.1 Paper I

- Grx2 comprised 80 % of the catalytic activity of the glutaredoxins in the cell, when measured by the classical HED assay.

- Grx2 has a protective role in the defense against oxidative stresses. The relevant null mutant had very high levels of protein carbonylation after treatment of the cells with hydrogen peroxide.

- Grx1 could act as a disulfide bond-forming enzyme under oxidizing conditions. This has been reported for the thioredoxin previously, but not for any glutaredoxin.

3.1.1.2 Paper II

- Five sandwich ELISAs were developed, for Grx1, Grx2, Grx3, Trx1 and Trx2 in *E. coli*. The assays were shown to be highly specific and sensitive.

- Levels of Grx2 and Grx3 were found to be very abundant in the cell, with Grx2 reaching as high as to 10 μg/mg of total soluble protein.

- Grx1 and to a less extend Trx1, were shown to be the main hydrogen donors for ribonucleotide reductase.

3.1.1.3 Paper III

- As previously shown for Trx1, Grx2 was elevated at the stationary phase of growth. Furthermore, ppGpp and RpoS regulated the expression of Grx2.

- Grx2 was positively regulated by osmotic upshock, and downregulated by cAMP. These changes might be a direct effect on Grx2 or an indirect via σ^s.

3.1.1.4 Paper IV

- PAPS reductase is glutathionylated *in vitro* and *in vivo*, and its activity is redox regulated by the glutaredoxins.

3.1.1.5 Paper V

- A novel glutaredoxin, Grx4, was discovered, belonging to the category of monothiol glutaredoxins, having the active site CGFS.

- Grx4 seems having a thioredoxin/glutaredoxin fold, but with a higher α-helical content (CD spectra).

- Grx4 lacks activity in the HED assay, but is active in the reconstitution of the Fe/S cluster protein FNR.

- Grx4 is likely to be regulated by ppGpp in an RpoS independent manner, but only at the stationary phase of growth.

4 FUTURE PERSPECTIVES

Findings presented in this thesis have raised several important questions concerning the role and function of different proteins of the thioredoxin superfamily. Listed below are some questions that I would like to address in the future.

4.1 EXAMINE REGULATION OF GLUTAREDOXINS AT TRANSCRIPTIONAL LEVEL

Our analysis on the regulation of Grx2 and Grx4 have so fare only been performed at the protein level. To better understand the mechanism, we are planning to perform quantitative mRNA measurements of Grx2 and Grx4 to really show that there is a transcriptional regulation. In addition we would like to do *in vitro* transcription experiments with different forms of *E. coli* RNA polymerase.

4.2 IDENTIFICATION OF CANDIDATE SUBSTRATES FOR GRX2, GRX3 AND GRX4

We recently found that Grx2, Grx3 and Grx4 are highly abundant in cells, with Grx2 reaching up to 10 μg/mg (one per cent) of total soluble protein while contributing to more than 80 % of total GSH-oxidoreductase activity (HED assay) in *E. coli* crude extracts. However it is only Grx1 that has been thoroughly characterized in terms of specific electron acceptors. We therefore want to characterize the unknown substrates for these glutaredoxins using the following methods:

(i) Via monothiol glutaredoxin mutants.

Overexpression of a monothiol glutaredoxin species fused to a His-tag will lead to formation of a stable complex of monothiol glutaredoxin and its substrate(s). The complex will be purified using an affinity matrix for the His-tag. Reduction with DTT will release the glutaredoxin partner, which will finally be identified with electron spray mass spectrometry or N-terminal sequencing.

(ii) Via the identification of substrates after reduction-alkylation.

Crude lysates will be alkylated and then treated with reduced glutaredoxin. A new alkylation will follow with a fluorescent alkylator. Lysates will be analyzed in 2D gels and samples treated with glutaredoxins will be compared with the non-treated controls. Fluorescent spots in the glutaredoxin-treated samples (good candidates for glutaredoxin substrates) will be analyzed by electron spray mass spectrometry or N-terminal sequencing.

With the second method, we will also be able to identify *in vivo* glutathionylated proteins. Glutathionylation is now a day believed to play an important role in protein regulation, and over 2 % of the total protein concentration is glutathionylated in *E. coli* under normal conditions.

4.3 STRUCTURAL DETERMINATION OF GRX4

A structure of a monothiol glutaredoxin is currently lacking. Such a structure would provide information on the folding similarities between classical dithiol and monothiol glutaredoxin isoforms. It will also contribute to a better understanding of the monothiol mechanism and the substrate specificity of monothiol glutaredoxin species. Therefore, we are currently working on the structural determination of Grx4 in collaboration with Malin Fladvad and Maria Sunnerhagen (Molecular Biophysics, MBB).

4.4 NRDH LEVELS AND REGULATION

NrdH is a protein with thioredoxin like enzymatic properties, but a structure similar to *E. coli* Grx3. Little is known today about its *in vivo* function and regulation of expression. Therefore, we have raised polyclonal antibodies against NrdH and will set up a specific ELISA method to determine the actual protein levels. We will also measure the levels of NrdH after growth under various conditions and in different genetic backgrounds to elucidate its specific function *in vivo*.

4.5 GLUTAREDOXIN LEVELS AND FUNCTIONS UNDER ANAEROBIC CONDITIONS

There is considerable evidence about the levels and function of glutaredoxins under aerobic conditions. We would therefore like to measure the glutaredoxin levels in different genetic backgrounds under strictly anaerobic conditions. This might help to elucidate functional differences between the glutaredoxins.

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6 REFERENCES

Agorio, A., Chalar, C., Cardozo, S., and Salinas, G. (2003). Alternative mRNAs arising from trans-splicing code for mitochondrial and cytosolic variants of Echinococcus granulosus thioredoxin glutathione reductase. J Biol Chem *21*, [epub ahead of print].

Ahn, B. Y., and Moss, B. (1992). Glutaredoxin homolog encoded by vaccinia virus is a virion-associated enzyme with thioltransferase and dehydroascorbate reductase activities. Proc Natl Acad Sci U S A *89*, 7060-7064.

Ajouz, B., Berrier, C., Besnard, M., Martinac, B., and Ghazi, A. (2000). Contributions of the different extramembranous domains of the mechanosensitive ion channel MscL to its response to membrane tension. J Biol Chem *275*, 1015-1022.

Ajouz, B., Berrier, C., Garrigues, A., Besnard, M., and Ghazi, A. (1998). Release of thioredoxin via the mechanosensitive channel MscL during osmotic downshock of Escherichia coli cells. J Biol Chem *273*, 26670-26674.

Amabile-Cuevas, C. F., and Demple, B. (1991). Molecular characterization of the soxRS genes of Escherichia coli: two genes control a superoxide stress regulon. Nucleic Acids Res *19*, 4479-4484.

Andersen, C. L., Matthey-Dupraz, A., Missiakas, D., and Raina, S. (1997). A new Escherichia coli gene, dsbG, encodes a periplasmic protein involved in disulphide bond formation, required for recycling DsbA/DsbB and DsbC redox proteins. Mol Microbiol *26*, 121-132.

Apontoweil, P., and Berends, W. (1975). Glutathione biosynthesis in Escherichia coli K 12. Properties of the enzymes and regulation. Biochim Biophys Acta *399*, 1-9.

Apontoweil, P., and Berends, W. (1975). Isolation and initial characterization of glutathione-deficient mutants of Escherichia coli K 12. Biochim Biophys Acta *399*, 10-22.

Arner, E. S., and Holmgren, A. (2000). Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem *267*, 6102-6109.

Arner, E. S., Nakamura, H., Sasada, T., Yodoi, J., Holmgren, A., and Spyrou, G. (2001). Analysis of the inhibition of mammalian thioredoxin, thioredoxin reductase, and glutaredoxin by cis-diamminedichloroplatinum (II) and its major metabolite, the glutathione-platinum complex. Free Radic Biol Med *31*, 1170-1178.

Arnold, C. N., McElhanon, J., Lee, A., Leonhart, R., and Siegele, D. A. (2001). Global analysis of Escherichia coli gene expression during the acetate- induced acid tolerance response. J Bacteriol *183*, 2178-2186.

Arscott, L. D., Gromer, S., Schirmer, R. H., Becker, K., and Williams, C. H., Jr. (1997). The mechanism of thioredoxin reductase from human placenta is similar to the mechanisms of lipoamide dehydrogenase and glutathione reductase and is distinct from the mechanism of thioredoxin reductase from Escherichia coli. Proc Natl Acad Sci U S A *94*, 3621-3626.

Åslund, F., Berndt, K. D., and Holmgren, A. (1997). Redox potentials of glutaredoxins and other thiol-disulfide oxidoreductases of the thioredoxin superfamily determined by direct protein-protein redox equilibria. J Biol Chem *272*, 30780-30786.

Åslund, F., Ehn, B., Miranda-Vizuete, A., Pueyo, C., and Holmgren, A. (1994). Two additional glutaredoxins exist in Escherichia coli: glutaredoxin 3 is a hydrogen donor

for ribonucleotide reductase in a thioredoxin/glutaredoxin 1 double mutant. Proc Natl Acad Sci U S A *91*, 9813-9817.

Åslund, F., Ehn, B., Miranda-Vizuete, A., Pueyo, C., and Holmgren, A. (1994). Two additional glutaredoxins exist in Escherichia coli: glutaredoxin 3 is a hydrogen donor for ribonucleotide reductase in a thioredoxin/glutaredoxin 1 double mutant. Proc. Natl. Acad. Sci. U.S.A. *91*, 9813-9817.

Åslund, F., Nordstrand, K., Berndt, K. D., Nikkola, M., Bergman, T., Ponstingl, H., Jornvall, H., Otting, G., and Holmgren, A. (1996). Glutaredoxin-3 from Escherichia coli. Amino acid sequence, 1H AND 15N NMR assignments, and structural analysis. J Biol Chem *271*, 6736-6745.

Åslund, F., Nordstrand, K., Berndt, K. D., Nikkola, M., Bergman, T., Ponstingl, H., Jörnvall, H., Otting, G., and Holmgren, A. (1996). Glutaredoxin-3 from Escherichia coli. Amino acid sequence, 1H AND 15N NMR assignments, and structural analysis. J Biol Chem *271*, 6736-6745.

Åslund, F., Zheng, M., Beckwith, J., and Storz, G. (1999). Regulation of the OxyR transcription factor by hydrogen peroxide and the cellular thiol-disulfide status. Proc Natl Acad Sci U S A *96*, 6161-6165.

Audia, J. P., Webb, C. C., and Foster, J. W. (2001). Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. Int J Med Microbiol *291*, 97-106.

Autret, S., Levine, A., Vannier, F., Fujita, Y., and Seror, S. J. (1999). The replication checkpoint control in Bacillus subtilis: identification of a novel RTP-binding sequence essential for the replication fork arrest after induction of the stringent response. Mol Microbiol *31*, 1665-1679.

Bader, M. W., Hiniker, A., Regeimbal, J., Goldstone, D., Haebel, P. W., Riemer, J., Metcalf, P., and Bardwell, J. C. (2001). Turning a disulfide isomerase into an oxidase: DsbC mutants that imitate DsbA. Embo J *20*, 1555-1562.

Bader, M. W., Xie, T., Yu, C. A., and Bardwell, J. C. (2000). Disulfide bonds are generated by quinone reduction. J Biol Chem *275*, 26082-26088.

Bandyopadhyay, S., and Gronostajski, R. M. (1994). Identification of a conserved oxidation-sensitive cysteine residue in the NFI family of DNA-binding proteins. J Biol Chem *269*, 29949-29955.

Bandyopadhyay, S., Starke, D. W., Mieyal, J. J., and Gronostajski, R. M. (1998). Thioltransferase (glutaredoxin) reactivates the DNA-binding activity of oxidation-inactivated nuclear factor I. J Biol Chem *273*, 392-397.

Bardwell, J. C., Lee, J. O., Jander, G., Martin, N., Belin, D., and Beckwith, J. (1993). A pathway for disulfide bond formation in vivo. Proc Natl Acad Sci U S A *90*, 1038-1042.

Bardwell, J. C., McGovern, K., and Beckwith, J. (1991). Identification of a protein required for disulfide bond formation in vivo. Cell *67*, 581-589.

Barrett, W. C., DeGnore, J. P., Konig, S., Fales, H. M., Keng, Y. F., Zhang, Z. Y., Yim, M. B., and Chock, P. B. (1999). Regulation of PTP1B via glutathionylation of the active site cysteine 215. Biochemistry *38*, 6699-6705.

Bartlett, M. S., Gaal, T., Ross, W., and Gourse, R. L. (1998). RNA polymerase mutants that destabilize RNA polymerase-promoter complexes alter NTP-sensing by rrn P1 promoters. J Mol Biol *279*, 331-345.

Battistoni, A., Mazzetti, A. P., and Rotilio, G. (1999). In vivo formation of Cu,Zn superoxide dismutase disulfide bond in Escherichia coli. FEBS Lett *443*, 313-316.

Becker-Hapak, M., and Eisenstark, A. (1995). Role of rpoS in the regulation of glutathione oxidoreductase (gor) in Escherichia coli. FEMS Microbiol Lett *134*, 39-44.

Berendt, U., Haverkamp, T., Prior, A., and Schwenn, J. D. (1995). Reaction mechanism of thioredoxin: 3'-phospho-adenylylsulfate reductase investigated by site-directed mutagenesis. Eur J Biochem *233*, 347-356.

Berrier, C., Coulombe, A., Szabo, I., Zoratti, M., and Ghazi, A. (1992). Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. Eur J Biochem *206*, 559-565.

Berrier, C., Garrigues, A., Richarme, G., and Ghazi, A. (2000). Elongation factor Tu and DnaK are transferred from the cytoplasm to the periplasm of Escherichia coli during osmotic downshock presumably via the mechanosensitive channel mscL. J Bacteriol *182*, 248-251.

Bessette, P. H., Åslund, F., Beckwith, J., and Georgiou, G. (1999). Efficient folding of proteins with multiple disulfide bonds in the Escherichia coli cytoplasm. Proc Natl Acad Sci U S A *96*, 13703-13708.

Bessette, P. H., Cotto, J. J., Gilbert, H. F., and Georgiou, G. (1999). In vivo and in vitro function of the Escherichia coli periplasmic cysteine oxidoreductase DsbG. J Biol Chem *274*, 7784-7792.

Bianchi, A. A., and Baneyx, F. (1999). Hyperosmotic shock induces the sigma32 and sigmaE stress regulons of Escherichia coli. Mol Microbiol *34*, 1029-1038.

Bjornstedt, M., Xue, J., Huang, W., Akesson, B., and Holmgren, A. (1994). The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. J Biol Chem *269*, 29382-29384.

Blount, P., Sukharev, S. I., Moe, P. C., Schroeder, M. J., Guy, H. R., and Kung, C. (1996). Membrane topology and multimeric structure of a mechanosensitive channel protein of Escherichia coli. Embo J *15*, 4798-4805.

Board, P. G., Coggan, M., Chelvanayagam, G., Easteal, S., Jermiin, L. S., Schulte, G. K., Danley, D. E., Hoth, L. R., Griffor, M. C., Kamath, A. V., Rosner, M. H., Chrunyk, B. A., Perregaux, D. E., Gabel, C. A., Geoghegan, K. F., and Pandit, J. (2000). Identification, characterization, and crystal structure of the Omega class glutathione transferases. J Biol Chem *275*, 24798-24806.

Borges, C. R., Geddes, T., Watson, J. T., and Kuhn, D. M. (2002). Dopamine biosynthesis is regulated by S-glutathionylation. Potential mechanism of tyrosine hydroxylase inhibition during oxidative stress. J Biol Chem *277*, 48295-48302.

Borges, C. R., Geddes, T. J., Watson, J. T., and Kuhn, D. M. (2002). Tyrosine hydroxylase is regulated by S-glutathionylation: Potential mechanism of dopamine synthesis inhibition under oxidative stress. J Biol Chem *9*, 9.

Bradley, T. M., Hidalgo, E., Leautaud, V., Ding, H., and Demple, B. (1997). Cysteine-to-alanine replacements in the Escherichia coli SoxR protein and the role of the [2Fe-2S] centers in transcriptional activation. Nucleic Acids Res *25*, 1469-1475.

Brown, N. C., and Reichard, P. (1969). Role of effector binding in allosteric control of ribonucleoside diphosphate reductase. J Mol Biol *46*, 39-55.

Buhl, R., Jaffe, H. A., Holroyd, K. J., Wells, F. B., Mastrangeli, A., Saltini, C., Cantin, A. M., and Crystal, R. G. (1989). Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. Lancet *2*, 1294-1298.

Bushweller, J. H., Åslund, F., Wuthrich, K., and Holmgren, A. (1992). Structural and functional characterization of the mutant Escherichia coli glutaredoxin (C14----S) and its mixed disulfide with glutathione. Biochemistry *31*, 9288-9293.

Bushweller, J. H., Billeter, M., Holmgren, A., and Wuthrich, K. (1994). The nuclear magnetic resonance solution structure of the mixed disulfide between Escherichia coli glutaredoxin(C14S) and glutathione. J Mol Biol *235*, 1585-1597.

Carlioz, A., and Touati, D. (1986). Isolation of superoxide dismutase mutants in Escherichia coli: is superoxide dismutase necessary for aerobic life? Embo J *5*, 623-630.

Carmel-Harel, O., and Storz, G. (2000). Roles of the glutathione- and thioredoxin-dependent reduction systems in the Escherichia coli and saccharomyces cerevisiae responses to oxidative stress. Annu Rev Microbiol *54*, 439-461.

Cashel, M., Gentry, D. R., Hernandez, V. J., and D., V. (1996). Eschericha coli and Salmonella, 2nd Ed, Volume 1, Neidhardt, ed.: ASM press).

Castanie-Cornet, M. P., and Foster, J. W. (2001). Escherichia coli acid resistance: cAMP receptor protein and a 20 bp cis- acting sequence control pH and stationary phase expression of the gadA and gadBC glutamate decarboxylase genes. Microbiology *147*, 709-715.

Castanie-Cornet, M. P., Penfound, T. A., Smith, D., Elliott, J. F., and Foster, J. W. (1999). Control of acid resistance in Escherichia coli. J Bacteriol *181*, 3525-3535.

Cha, M. K., Kim, H. K., and Kim, I. H. (1996). Mutation and Mutagenesis of thiol peroxidase of Escherichia coli and a new type of thiol peroxidase family. J Bacteriol *178*, 5610-5614.

Cha, M. K., Kim, H. K., and Kim, I. H. (1995). Thioredoxin-linked "thiol peroxidase" from periplasmic space of Escherichia coli. J Biol Chem *270*, 28635-28641.

Chae, H. Z., Chung, S. J., and Rhee, S. G. (1994). Thioredoxin-dependent peroxide reductase from yeast. J Biol Chem *269*, 27670-27678.

Chae, H. Z., Robison, K., Poole, L. B., Church, G., Storz, G., and Rhee, S. G. (1994). Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. Proc Natl Acad Sci U S A *91*, 7017-7021.

Chamberlin, M. (1974). Isolation and characterization of prototrophic mutants of Escherichia coli unable to support the intracellular growth of T7. J Virol *14*, 509-516.

Chasseaud, L. F. (1979). The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv Cancer Res *29*, 175-274.

Chatterji, D., Fujita, N., and Ishihama, A. (1998). The mediator for stringent control, ppGpp, binds to the beta-subunit of Escherichia coli RNA polymerase. Genes Cells *3*, 279-287.

Cho, Y. W., Kim, J. C., Jin, C. D., Han, T. J., and Lim, C. J. (1998). Thioltransferase from Arabidopsis thaliana seed: purification to homogeneity and characterization. Mol Cells *8*, 550-555.

Choi, H., Kim, S., Mukhopadhyay, P., Cho, S., Woo, J., Storz, G., and Ryu, S. (2001). Structural basis of the redox switch in the OxyR transcription factor. Cell *105*, 103-113.

Christman, M. F., Morgan, R. W., Jacobson, F. S., and Ames, B. N. (1985). Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in Salmonella typhimurium. Cell *41*, 753-762.

Christman, M. F., Storz, G., and Ames, B. N. (1989). OxyR, a positive regulator of hydrogen peroxide-inducible genes in Escherichia coli and Salmonella typhimurium, is homologous to a family of bacterial regulatory proteins. Proc Natl Acad Sci U S A *86*, 3484-3488.

Claiborne, A., and Fridovich, I. (1979). Purification of the o-dianisidine peroxidase from Escherichia coli B. Physicochemical characterization and analysis of its dual catalatic and peroxidatic activities. J Biol Chem *254*, 4245-4252.

Claiborne, A., Malinowski, D. P., and Fridovich, I. (1979). Purification and characterization of hydroperoxidase II of Escherichia coli B. J Biol Chem *254*, 11664-11668.

Collet, J. F., Riemer, J., Bader, M. W., and Bardwell, J. C. (2002). Reconstitution of a disulfide isomerization system. J Biol Chem *277*, 26886-26892.

Collinson, E. J., Wheeler, G. L., Garrido, E. O., Avery, A. M., Avery, S. V., and Grant, C. M. (2002). The yeast glutaredoxins are active as glutathione peroxidases. J Biol Chem *277*, 16712-16717.

Cotgreave, I. A., Gerdes, R., Schuppe-Koistinen, I., and Lind, C. (2002). S-glutathionylation of glyceraldehyde-3-phosphate dehydrogenase: role of thiol oxidation and catalysis by glutaredoxin. Methods Enzymol *348*, 175-182.

Cotgreave, I. A., and Gerdes, R. G. (1998). Recent trends in glutathione biochemistry--glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? Biochem Biophys Res Commun *242*, 1-9.

Dailey, F. E., and Berg, H. C. (1993). Mutants in disulfide bond formation that disrupt flagellar assembly in Escherichia coli. Proc Natl Acad Sci U S A *90*, 1043-1047.

Daily, D., Vlamis-Gardikas, A., Offen, D., Mittelman, L., Melamed, E., Holmgren, A., and Barzilai, A. (2001a). Glutaredoxin protects cerebellar granule neurons from dopamine-induced apoptosis by activating NF-kappa B via Ref-1. J Biol Chem *276*, 1335-1344.

Daily, D., Vlamis-Gardikas, A., Offen, D., Mittelman, L., Melamed, E., Holmgren, A., and Barzilai, A. (2001b). Glutaredoxin protects cerebellar granule neurons from

dopamine-induced apoptosis by dual activation of the ras-phosphoinositide 3-kinase and jun n-terminal kinase pathways. J Biol Chem *276*, 21618-21626.

Darby, N. J., Raina, S., and Creighton, T. E. (1998). Contributions of substrate binding to the catalytic activity of DsbC. Biochemistry *37*, 783-791.

Darke, P. L., Jordan, S. P., Hall, D. L., Zugay, J. A., Shafer, J. A., and Kuo, L. C. (1994). Dissociation and association of the HIV-1 protease dimer subunits: equilibria and rates. Biochemistry *33*, 98-105.

Davis, D. A., Dorsey, K., Wingfield, P. T., Stahl, S. J., Kaufman, J., Fales, H. M., and Levine, R. L. (1996). Regulation of HIV-1 protease activity through cysteine modification. Biochemistry *35*, 2482-2488.

Davis, D. A., Newcomb, F. M., Starke, D. W., Ott, D. E., Mieyal, J. J., and Yarchoan, R. (1997). Thioltransferase (glutaredoxin) is detected within HIV-1 and can regulate the activity of glutathionylated HIV-1 protease in vitro. J Biol Chem *272*, 25935-25940.

De Rosa, S. C., Zaretsky, M. D., Dubs, J. G., Roederer, M., Anderson, M., Green, A., Mitra, D., Watanabe, N., Nakamura, H., Tjioe, I., Deresinski, S. C., Moore, W. A., Ela, S. W., Parks, D., and Herzenberg, L. A. (2000). N-acetylcysteine replenishes glutathione in HIV infection. Eur J Clin Invest *30*, 915-929.

Debarbieux, L., and Beckwith, J. (1999). Electron avenue: pathways of disulfide bond formation and isomerization. Cell *99*, 117-119.

Debarbieux, L., and Beckwith, J. (2000). On the functional interchangeability, oxidant versus reductant, of members of the thioredoxin superfamily. J Bacteriol *182*, 723-727.

Debarbieux, L., and Beckwith, J. (1998). The reductive enzyme thioredoxin 1 acts as an oxidant when it is exported to the Escherichia coli periplasm. Proc Natl Acad Sci U S A *95*, 10751-10756.

Ding, H., and Demple, B. (1996). Glutathione-mediated destabilization in vitro of [2Fe-2S] centers in the SoxR regulatory protein. Proc Natl Acad Sci U S A *93*, 9449-5943.

Ding, H., and Demple, B. (1997). In vivo kinetics of a redox-regulated transcriptional switch. Proc Natl Acad Sci U S A *94*, 8445-9844.

Ding, H., and Demple, B. (1998). Thiol-mediated disassembly and reassembly of [2Fe-2S] clusters in the redox-regulated transcription factor SoxR. Biochemistry *37*, 17280-17286.

Ding, H., Hidalgo, E., and Demple, B. (1996). The redox state of the [2Fe-2S] clusters in SoxR protein regulates its activity as a transcription factor. J Biol Chem *271*, 33173-33175.

Eck, H. P., Gmunder, H., Hartmann, M., Petzoldt, D., Daniel, V., and Droge, W. (1989). Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol Chem Hoppe Seyler *370*, 101-108.

Edeling, M. A., Guddat, L. W., Fabianek, R. A., Thony-Meyer, L., and Martin, J. L. (2002). Structure of CcmG/DsbE at 1.14 A resolution: high-fidelity reducing activity in an indiscriminately oxidizing environment. Structure (Camb) *10*, 973-979.

Edman, J. C., Ellis, L., Blacher, R. W., Roth, R. A., and Rutter, W. J. (1985). Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin. Nature *317*, 267-270.

Eisenstark, A., Yallaly, P., Ivanova, A., and Miller, C. (1995). Genetic mechanisms involved in cellular recovery from oxidative stress. Arch Insect Biochem Physiol *29*, 159-173.

Eklund, H., Gleason, F. K., and Holmgren, A. (1991). Structural and functional relations among thioredoxins of different species. Proteins *11*, 13-28.

Eklund, H., Ingelman, M., Soderberg, B. O., Uhlin, T., Nordlund, P., Nikkola, M., Sonnerstam, U., Joelson, T., and Petratos, K. (1992). Structure of oxidized bacteriophage T4 glutaredoxin (thioredoxin). Refinement of native and mutant proteins. J Mol Biol *228*, 596-618.

Eklund, H., Uhlin, U., Farnegardh, M., Logan, D. T., and Nordlund, P. (2001). Structure and function of the radical enzyme ribonucleotide reductase. Prog Biophys Mol Biol *77*, 177-268.

Eliasson, R., Pontis, E., Jordan, A., and Reichard, P. (1996). Allosteric regulation of the third ribonucleotide reductase (NrdEF enzyme) from enterobacteriaceae. J Biol Chem *271*, 26582-26587.

Epp, O., Ladenstein, R., and Wendel, A. (1983). The refined structure of the selenoenzyme glutathione peroxidase at 0.2- nm resolution. Eur J Biochem *133*, 51-69.

Fabianek, R. A., Hennecke, H., and Thony-Meyer, L. (1998). The active-site cysteines of the periplasmic thioredoxin-like protein CcmG of Escherichia coli are important but not essential for cytochrome c maturation in vivo. J Bacteriol *180*, 1947-1950.

Fabianek, R. A., Hennecke, H., and Thony-Meyer, L. (2000). Periplasmic protein thiol:disulfide oxidoreductases of Escherichia coli. FEMS Microbiol Rev *24*, 303-316.

Fabianek, R. A., Hofer, T., and Thony-Meyer, L. (1999). Characterization of the Escherichia coli CcmH protein reveals new insights into the redox pathway required for cytochrome c maturation. Arch Microbiol *171*, 92-100.

Fabianek, R. A., Huber-Wunderlich, M., Glockshuber, R., Kunzler, P., Hennecke, H., and Thony-Meyer, L. (1997). Characterization of the Bradyrhizobium japonicum CycY protein, a membrane-anchored periplasmic thioredoxin that may play a role as a reductant in the biogenesis of c-type cytochromes. J Biol Chem *272*, 4467-4473.

Ferguson, G. P., McLaggan, D., and Booth, I. R. (1995). Potassium channel activation by glutathione-S-conjugates in Escherichia coli: protection against methylglyoxal is mediated by cytoplasmic acidification. Mol Microbiol *17*, 1025-1033.

Fernandez, L. A., and de Lorenzo, V. (2001). Formation of disulphide bonds during secretion of proteins through the periplasmic-independent type I pathway. Mol Microbiol *40*, 332-346.

Fomenko, D. E., and Gladyshev, V. N. (2002). CxxS: fold-independent redox motif revealed by genome-wide searches for thiol/disulfide oxidoreductase function. Protein Sci *11*, 2285-2296.

Fontecave, M., Nordlund, P., Eklund, H., and Reichard, P. (1992). The redox centers of ribonucleotide reductase of Escherichia coli. Adv Enzymol Relat Areas Mol Biol *65*, 147-183.

Fuchs, J. A., and Warner, H. R. (1975). Isolation of an Escherichia coli mutant deficient in glutathione synthesis. J Bacteriol *124*, 140-148.

Gajiwala, K. S., and Burley, S. K. (2000). HDEA, a periplasmic protein that supports acid resistance in pathogenic enteric bacteria. J Mol Biol *295*, 605-612.

Galiazzo, F., Schiesser, A., and Rotilio, G. (1987). Glutathione peroxidase in yeast. Presence of the enzyme and induction by oxidative conditions. Biochem Biophys Res Commun *147*, 1200-1205.

Gan, Z. R., Polokoff, M. A., Jacobs, J. W., and Sardana, M. K. (1990). Complete amino acid sequence of yeast thioltransferase (glutaredoxin). Biochem Biophys Res Commun *168*, 944-951.

Gardner, P. R., and Fridovich, I. (1993). Effect of glutathione on aconitase in Escherichia coli. Arch Biochem Biophys *301*, 98-102.

Gaudu, P., and Weiss, B. (1996). SoxR, a [2Fe-2S] transcription factor, is active only in its oxidized form. Proc Natl Acad Sci U S A *93*, 10094-10098.

Ghezzi, P., Romines, B., Fratelli, M., Eberini, I., Gianazza, E., Casagrande, S., Laragione, T., Mengozzi, M., and Herzenberg, L. A. (2002). Protein glutathionylation: coupling and uncoupling of glutathione to protein thiol groups in lymphocytes under oxidative stress and HIV infection. Mol Immunol *38*, 773-780.

Gilbert, H. F. (1984). Redox control of enzyme activities by thiol/disulfide exchange. Methods Enzymol *107*, 330-351.

Ginsberg, S. D., Hemby, S. E., Lee, V. M., Eberwine, J. H., and Trojanowski, J. Q. (2000). Expression profile of transcripts in Alzheimer's disease tangle-bearing CA1 neurons. Ann Neurol *48*, 77-87.

Gladyshev, V. N., Jeang, K. T., and Stadtman, T. C. (1996). Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. Proc Natl Acad Sci U S A *93*, 6146-6151.

Gladysheva, T. B., Oden, K. L., and Rosen, B. P. (1994). Properties of the arsenate reductase of plasmid R773. Biochemistry *33*, 7288-7293.

Gonzalez Porque, P., Baldesten, A., and Reichard, P. (1970). The involvement of the thioredoxin system in the reduction of methionine sulfoxide and sulfate. J Biol Chem *245*, 2371-2374.

Gonzalez-Flecha, B., and Demple, B. (1997). Transcriptional regulation of the Escherichia coli oxyR gene as a function of cell growth. J Bacteriol *179*, 6181-6186.

Gourse, R. L., Gaal, T., Aiyar, S. E., Barker, M. M., Estrem, S. T., Hirvonen, C. A., and Ross, W. (1998). Strength and regulation without transcription factors: lessons from bacterial rRNA promoters. Cold Spring Harb Symp Quant Biol *63*, 131-139.

Grant, C. M. (2001). Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions. Mol Microbiol *39*, 533-541.

Grant, C. M., Luikenhuis, S., Beckhouse, A., Soderbergh, M., and Dawes, I. W. (2000). Differential regulation of glutaredoxin gene expression in response to stress conditions in the yeast Saccharomyces cerevisiae. Biochim Biophys Acta *1490*, 33-42.

Gravina, S. A., and Mieyal, J. J. (1993). Thioltransferase is a specific glutathionyl mixed disulfide oxidoreductase. Biochemistry *32*, 3368-3376.

Greenberg, J. T., and Demple, B. (1986). Glutathione in Escherichia coli is dispensable for resistance to H2O2 and gamma radiation. J Bacteriol *168*, 1026-1029.

Greenberg, J. T., Monach, P., Chou, J. H., Josephy, P. D., and Demple, B. (1990). Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in Escherichia coli. Proc Natl Acad Sci U S A *87*, 6181-6185.

Guilhot, C., Jander, G., Martin, N. L., and Beckwith, J. (1995). Evidence that the pathway of disulfide bond formation in Escherichia coli involves interactions between the cysteines of DsbB and DsbA. Proc Natl Acad Sci U S A *92*, 9895-9859.

Gvakharia, B. O., Koonin, E. K., and Mathews, C. K. (1996). Vaccinia virus G4L gene encodes a second glutaredoxin. Virology *226*, 408-411.

Harrop, S. J., DeMaere, M. Z., Fairlie, W. D., Reztsova, T., Valenzuela, S. M., Mazzanti, M., Tonini, R., Qiu, M. R., Jankova, L., Warton, K., Bauskin, A. R., Wu, W. M., Pankhurst, S., Campbell, T. J., Breit, S. N., and Curmi, P. M. (2001). Crystal structure of a soluble form of the intracellular chloride ion channel CLIC1 (NCC27) at 1.4-A resolution. J Biol Chem *276*, 44993-5000.

Haseltine, W. A., and Block, R. (1973). Synthesis of guanosine tetra- and pentaphosphate requires the presence of a codon-specific, uncharged transfer ribonucleic acid in the acceptor site of ribosomes. Proc Natl Acad Sci U S A *70*, 1564-1568.

Heiskanen, P., Taira, S., and Rhen, M. (1994). Role of rpoS in the regulation of Salmonella plasmid virulence (spv) genes. FEMS Microbiol Lett *123*, 125-130.

Hengge-Aronis, R., Lange, R., Henneberg, N., and Fischer, D. (1993). Osmotic regulation of rpoS-dependent genes in Escherichia coli. J Bacteriol *175*, 259-265.

Herrero, E., and Ros, J. (2002). Glutaredoxins and oxidative stress defense in yeast. Methods Enzymol *348*, 136-146.

Hersh, B. M., Farooq, F. T., Barstad, D. N., Blankenhorn, D. L., and Slonczewski, J. L. (1996). A glutamate-dependent acid resistance gene in Escherichia coli. J Bacteriol *178*, 3978-3981.

Herzenberg, L. A., De Rosa, S. C., Dubs, J. G., Roederer, M., Anderson, M. T., Ela, S. W., and Deresinski, S. C. (1997). Glutathione deficiency is associated with impaired survival in HIV disease. Proc Natl Acad Sci U S A *94*, 1967-1972.

Hibberd, K. A., Berget, P. B., Warner, H. R., and Fuchs, J. A. (1978). Role of glutathione in reversing the deleterious effects of a thiol- oxidizing agent in Escherichia coli. J Bacteriol *133*, 1150-1155.

Hidalgo, E., Bollinger, J. M., Jr., Bradley, T. M., Walsh, C. T., and Demple, B. (1995). Binuclear [2Fe-2S] clusters in the Escherichia coli SoxR protein and role of the metal centers in transcription. J Biol Chem *270*, 20908-209014.

Hidalgo, E., and Demple, B. (1994). An iron-sulfur center essential for transcriptional activation by the redox-sensing SoxR protein. Embo J *13*, 138-146.

Holmgren, A. (2000). Antioxidant function of thioredoxin and glutaredoxin systems. Antioxid Redox Signal *2*, 811-820.

Holmgren, A. (1979a). Glutathione-dependent synthesis of deoxyribonucleotides. Characterization of the enzymatic mechanism of Escherichia coli glutaredoxin. J Biol Chem *254*, 3672-3678.

Holmgren, A. (1979b). Glutathione-dependent synthesis of deoxyribonucleotides. Purification and characterization of glutaredoxin from Escherichia coli. J Biol Chem *254*, 3664-3671.

Holmgren, A. (1976). Hydrogen donor system for Escherichia coli ribonucleoside-diphosphate reductase dependent upon glutathione. Proc. Natl. Acad. Sci. U.S.A. *73*, 2275-2279.

Holmgren, A. (1989). Thioredoxin and glutaredoxin systems. J Biol Chem *264*, 13963-13966.

Holmgren, A. (1968). Thioredoxin. 6. The amino acid sequence of the protein from Escherichia coli B. Eur J Biochem *6*, 475-484.

Holmgren, A., and Åslund, F. (1995). Glutaredoxin. In Methods Enzymol, pp. 283-292.

Holmgren, A., Ohlsson, I., and Grankvist, M. L. (1978). Thioredoxin from Escherichia coli. Radioimmunological and enzymatic determinations in wild type cells and mutants defective in phage T7 DNA replication. J Biol Chem *253*, 430-436.

Holmgren, A., Soderberg, B. O., Eklund, H., and Branden, C. I. (1975). Three-dimensional structure of Escherichia coli thioredoxin-S2 to 2.8 A resolution. Proc Natl Acad Sci U S A *72*, 2305-2309.

Hoog, J. O., Jornvall, H., Holmgren, A., Carlquist, M., and Persson, M. (1983). The primary structure of Escherichia coli glutaredoxin. Distant homology with thioredoxins in a superfamily of small proteins with a redox-active cystine disulfide/cysteine dithiol. European Journal of Biochemistry *136*, 223-232.

Hopper, S., Johnson, R. S., Vath, J. E., and Biemann, K. (1989). Glutaredoxin from rabbit bone marrow. Purification, characterization, and amino acid sequence determined by tandem mass spectrometry. J Biol Chem *264*, 20438-20447.

Huang, T. T., Yasunami, M., Carlson, E. J., Gillespie, A. M., Reaume, A. G., Hoffman, E. K., Chan, P. H., Scott, R. W., and Epstein, C. J. (1997). Superoxide-mediated cytotoxicity in superoxide dismutase-deficient fetal fibroblasts. Arch Biochem Biophys *344*, 424-432.

Huber-Wunderlich, M., and Glockshuber, R. (1998). A single dipeptide sequence modulates the redox properties of a whole enzyme family. Fold Des *3*, 161-171.

Iizuka, M., Inoue, Y., Murata, K., and Kimura, A. (1989). Purification and some properties of glutathione S-transferase from Escherichia coli B. J Bacteriol *171*, 6039-6042.

Inaba, K., and Ito, K. (2002). Paradoxical redox properties of DsbB and DsbA in the protein disulfide- introducing reaction cascade. Embo J *21*, 2646-2654.

Inoue, Y., Matsuda, T., Sugiyama, K., Izawa, S., and Kimura, A. (1999). Genetic analysis of glutathione peroxidase in oxidative stress response of Saccharomyces cerevisiae. J Biol Chem *274*, 27002-27009.

Isakov, N., Witte, S., and Altman, A. (2000). PICOT-HD: a highly conserved protein domain that is often associated with thioredoxin and glutaredoxin modules. Trends Biochem Sci *25*, 537-539.

Jacobson, F. S., Morgan, R. W., Christman, M. F., and Ames, B. N. (1989). An alkyl hydroperoxide reductase from Salmonella typhimurium involved in the defense of DNA against oxidative damage. Purification and properties. J Biol Chem *264*, 1488-1496.

Jenney, F. E., Jr., Verhagen, M. F., Cui, X., and Adams, M. W. (1999). Anaerobic microbes: oxygen detoxification without superoxide dismutase. Science *286*, 306-309.

Jeong, W., Cha, M. K., and Kim, I. H. (2000). Thioredoxin-dependent hydroperoxide peroxidase activity of bacterioferritin comigratory protein (BCP) as a new member of the thiol- specific antioxidant protein (TSA)/Alkyl hydroperoxide peroxidase C (AhpC) family. J Biol Chem *275*, 2924-2930.

Jishage, M., and Ishihama, A. (1995). Regulation of RNA polymerase sigma subunit synthesis in Escherichia coli: intracellular levels of sigma 70 and sigma 38. J Bacteriol *177*, 6832-6835.

Johnson, T. E. (1990). Increased life-span of age-1 mutants in Caenorhabditis elegans and lower Gompertz rate of aging. Science *249*, 908-912.

Joly, J. C., and Swartz, J. R. (1997). In vitro and in vivo redox states of the Escherichia coli periplasmic oxidoreductases DsbA and DsbC. Biochemistry *36*, 10067-10072.

Jonda, S., Huber-Wunderlich, M., Glockshuber, R., and Mossner, E. (1999). Complementation of DsbA deficiency with secreted thioredoxin variants reveals the crucial role of an efficient dithiol oxidant for catalyzed protein folding in the bacterial periplasm. Embo J *18*, 3271-3281.

Jordan, A., Aslund, F., Pontis, E., Reichard, P., and Holmgren, A. (1997). Characterization of Escherichia coli NrdH. A glutaredoxin-like protein with a thioredoxin-like activity profile. J Biol Chem *272*, 18044-18050.

Jordan, A., Gibert, I., and Barbe, J. (1994). Cloning and sequencing of the genes from Salmonella typhimurium encoding a new bacterial ribonucleotide reductase. J Bacteriol *176*, 3420-3427.

Jordan, A., Pontis, E., Aslund, F., Hellman, U., Gibert, I., and Reichard, P. (1996). The ribonucleotide reductase system of Lactococcus lactis. Characterization of an NrdEF enzyme and a new electron transport protein. J Biol Chem *271*, 8779-8785.

Jordan, A., Pontis, E., Atta, M., Krook, M., Gibert, I., Barbe, J., and Reichard, P. (1994). A second class I ribonucleotide reductase in Enterobacteriaceae: characterization of the Salmonella typhimurium enzyme. Proc Natl Acad Sci U S A *91*, 12892-12896.

Jordan, A., and Reichard, P. (1998). Ribonucleotide reductases. Annu Rev Biochem *67*, 71-98.

Kallis, G. B., and Holmgren, A. (1980). Differential reactivity of the functional sulfhydryl groups of cysteine- 32 and cysteine-35 present in the reduced form of thioredoxin from Escherichia coli. J Biol Chem *255*, 10261-10265.

Kamitani, S., Akiyama, Y., and Ito, K. (1992). Identification and characterization of an Escherichia coli gene required for the formation of correctly folded alkaline phosphatase, a periplasmic enzyme. Embo J *11*, 57-62.

Katti, S. K., Robbins, A. H., Yang, Y., and Wells, W. W. (1995). Crystal structure of thioltransferase at 2.2 A resolution. Protein Sci *4*, 1998-2005.

Kelley, J. J., 3rd, and Bushweller, J. H. (1998). 1H, 13C and 15N NMR resonance assignments of vaccinia glutaredoxin-1 in the fully reduced form. J Biomol NMR *12*, 353-355.

Kemmink, J., Darby, N. J., Dijkstra, K., Nilges, M., and Creighton, T. E. (1996). Structure determination of the N-terminal thioredoxin-like domain of protein disulfide isomerase using multidimensional heteronuclear 13C/15N NMR spectroscopy. Biochemistry *35*, 7684-7691.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461-464.

Kim, K., Kim, I. H., Lee, K. Y., Rhee, S. G., and Stadtman, E. R. (1988). The isolation and purification of a specific "protector" protein which inhibits enzyme inactivation by a thiol/Fe(III)/O2 mixed-function oxidation system. J Biol Chem *263*, 4704-4711.

Kim, S. O., Merchant, K., Nudelman, R., Beyer, W. F., Jr., Keng, T., DeAngelo, J., Hausladen, A., and Stamler, J. S. (2002). OxyR: a molecular code for redox-related signaling. Cell *109*, 383-396.

Kishigami, S., and Ito, K. (1996). Roles of cysteine residues of DsbB in its activity to reoxidize DsbA, the protein disulphide bond catalyst of Escherichia coli. Genes Cells *1*, 201-208.

Klatt, P., and Lamas, S. (2000). Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur J Biochem *267*, 4928-4944.

Klatt, P., Molina, E. P., and Lamas, S. (1999). Nitric oxide inhibits c-Jun DNA binding by specifically targeted S- glutathionylation. J Biol Chem *274*, 15857-15864.

Kodym, R., Calkins, P., and Story, M. (1999). The cloning and characterization of a new stress response protein. A mammalian member of a family of theta class glutathione s-transferase- like proteins. J Biol Chem *274*, 5131-5137.

Kowarz, L., Coynault, C., Robbe-Saule, V., and Norel, F. (1994). The Salmonella typhimurium katF (rpoS) gene: cloning, nucleotide sequence, and regulation of spvR and spvABCD virulence plasmid genes. J Bacteriol *176*, 6852-6860.

Krause, G., Lundstrom, J., Barea, J. L., Pueyo de la Cuesta, C., and Holmgren, A. (1991). Mimicking the active site of protein disulfide-isomerase by substitution of proline 34 in Escherichia coli thioredoxin. J Biol Chem *266*, 9494-9500.

Krone, F. A., Westphal, G., and Schwenn, J. D. (1991). Characterisation of the gene cysH and of its product phospho- adenylylsulphate reductase from Escherichia coli. Mol Gen Genet *225*, 314-319.

Kumar, S., and Holmgren, A. (1999). Induction of thioredoxin, thioredoxin reductase and glutaredoxin activity in mouse skin by TPA, a calcium ionophore and other tumor promoters. Carcinogenesis *20*, 1761-1767.

Kuroda, A., Murphy, H., Cashel, M., and Kornberg, A. (1997). Guanosine tetra- and pentaphosphate promote accumulation of inorganic polyphosphate in Escherichia coli. J Biol Chem *272*, 21240-21243.

Kuroda, A., Tanaka, S., Ikeda, T., Kato, J., Takiguchi, N., and Ohtake, H. (1999). Inorganic polyphosphate kinase is required to stimulate protein degradation and for adaptation to amino acid starvation in Escherichia coli. Proc Natl Acad Sci U S A *96*, 14264-14269.

Kvint, K., Farewell, A., and Nystrom, T. (2000). RpoS-dependent Promoters Require Guanosine Tetraphosphate for Induction Even in the Presence of High Levels of sigma s. J Biol Chem *275*, 14795-14798.

Landis, L., Xu, J., and Johnson, R. C. (1999). The cAMP receptor protein CRP can function as an osmoregulator of transcription in Escherichia coli. Genes Dev *13*, 3081-3091.

Lange, R., and Hengge-Aronis, R. (1994). The cellular concentration of the sigma S subunit of RNA polymerase in Escherichia coli is controlled at the levels of transcription, translation, and protein stability. Genes Dev *8*, 1600-1612.

Lange, R., and Hengge-Aronis, R. (1991). Identification of a central regulator of stationary-phase gene expression in Escherichia coli. Mol Microbiol *5*, 49-59.

Laurent, T. C., Moore, E. C., Reichard, P. (1964). Enzymatic synthesis of deoxyribonucleotides. J. Biol. Chem. *239*, 3436-3444.

Lee, I. S., Lin, J., Hall, H. K., Bearson, B., and Foster, J. W. (1995). The stationary-phase sigma factor sigma S (RpoS) is required for a sustained acid tolerance response in virulent Salmonella typhimurium. Mol Microbiol *17*, 155-167.

Lennon, B. W., Williams, C. H., Jr., and Ludwig, M. L. (1999). Crystal structure of reduced thioredoxin reductase from Escherichia coli: structural flexibility in the isoalloxazine ring of the flavin adenine dinucleotide cofactor. Protein Sci *8*, 2366-2379.

Lennon, B. W., Williams, C. H., Jr., and Ludwig, M. L. (2000). Twists in catalysis: alternating conformations of Escherichia coli thioredoxin reductase. Science *289*, 1190-1194.

Licht, S., Gerfen, G. J., and Stubbe, J. (1996). Thiyl radicals in ribonucleotide reductases. Science *271*, 477-481.

Lillig, C. H., Prior, A., Schwenn, J. D., Åslund, F., Ritz, D., Vlamis-Gardikas, A., and Holmgren, A. (1999). New thioredoxins and glutaredoxins as electron donors of 3'-phosphoadenylylsulfate reductase. J Biol Chem *274*, 7695-7698.

Lim, C., Daws, T., Gerami-Nejad, M., and Fuchs, J. A. (2000). Growth-phase regulation of the Escherichia coli thioredoxin gene. Biochim Biophys Acta *1491*, 1-6.

Lim, C. J., Haller, B., and Fuchs, J. A. (1985). Thioredoxin is the bacterial protein encoded by fip that is required for filamentous bacteriophage f1 assembly. J Bacteriol *161*, 799-802.

Lin, J., Lee, I. S., Frey, J., Slonczewski, J. L., and Foster, J. W. (1995). Comparative analysis of extreme acid survival in Salmonella typhimurium, Shigella flexneri, and Escherichia coli. J Bacteriol *177*, 4097-4104.

Lin, J., Smith, M. P., Chapin, K. C., Baik, H. S., Bennett, G. N., and Foster, J. W. (1996). Mechanisms of acid resistance in enterohemorrhagic Escherichia coli. Appl Environ Microbiol *62*, 3094-3100.

Lin, Y. J., Seroude, L., and Benzer, S. (1998). Extended life-span and stress resistance in the Drosophila mutant methuselah. Science *282*, 943-946.

Lind, C., Gerdes, R., Hamnell, Y., Schuppe-Koistinen, I., von Lowenhielm, H., Holmgren, A., and Cotgreave, I. (2002). Identification of S-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteomic analysis. Arch Biochem Biophys *406*, 229.

Liochev, S. I., and Fridovich, I. (1997). A mechanism for complementation of the sodA sodB defect in Escherichia coli by overproduction of the rbo gene product (desulfoferrodoxin) from Desulfoarculus baarsii. J Biol Chem *272*, 25573-25575.

Loewen, P. C. (1979). Levels of glutathione in Escherichia coli. Can J Biochem *57*, 107-111.

Loewen, P. C., and Switala, J. (1986). Purification and characterization of catalase HPII from Escherichia coli K12. Biochem Cell Biol *64*, 638-646.

Loewen, P. C., Switala, J., and Triggs-Raine, B. L. (1985). Catalases HPI and HPII in Escherichia coli are induced independently. Arch Biochem Biophys *243*, 144-149.

Loewen, P. C., Triggs, B. L., George, C. S., and Hrabarchuk, B. E. (1985). Genetic mapping of katG, a locus that affects synthesis of the bifunctional catalase-peroxidase hydroperoxidase I in Escherichia coli. J Bacteriol *162*, 661-667.

Loewen, P. C., Triggs, B. L., Klassen, G. R., and Weiner, J. H. (1983). Identification and physical characterization of a Col E1 hybrid plasmid containing a catalase gene of Escherichia coli. Can J Biochem Cell Biol *61*, 1315-1321.

Lombard, M., Fontecave, M., Touati, D., and Niviere, V. (2000). Reaction of the desulfoferrodoxin from Desulfoarculus baarsii with superoxide anion. Evidence for a superoxide reductase activity. J Biol Chem *275*, 115-121.

Longo, V. D. (1999). Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells. Neurobiol Aging *20*, 479-486.

Longo, V. D., Gralla, E. B., and Valentine, J. S. (1996). Superoxide dismutase activity is essential for stationary phase survival in Saccharomyces cerevisiae. Mitochondrial production of toxic oxygen species in vivo. J Biol Chem *271*, 12275-12280.

Lucas, R. L., and Lee, C. A. (2000). Unravelling the mysteries of virulence gene regulation in Salmonella typhimurium. Mol Microbiol *36*, 1024-1033.

Luikenhuis, S., Perrone, G., Dawes, I. W., and Grant, C. M. (1998). The yeast Saccharomyces cerevisiae contains two glutaredoxin genes that are required for protection against reactive oxygen species. Mol Biol Cell *9*, 1081-1091.

Lundberg, M., Johansson, C., Chandra, J., Enoksson, M., Jacobsson, G., Ljung, J., Johansson, M., and Holmgren, A. (2001). Cloning and expression of a novel human glutaredoxin (Grx2) with mitochondrial and nuclear isoforms. J Biol Chem *276*, 26269-26275.

Lunn, C. A., Kathju, S., Wallace, B. J., Kushner, S. R., and Pigiet, V. (1984). Amplification and purification of plasmid-encoded thioredoxin from Escherichia coli K12. J Biol Chem *259*, 10469-10474.

Lunn, C. A., and Pigiet, V. P. (1982). Localization of thioredoxin from Escherichia coli in an osmotically sensitive compartment. J Biol Chem *257*, 11424-11430.

Luthman, M., Eriksson, S., Holmgren, A., and Thelander, L. (1979). Glutathione-dependent hydrogen donor system for calf thymus ribonucleoside-diphosphate reductase. Proc Natl Acad Sci U S A *76*, 2158-2162.

Maples, K. R., Kennedy, C. H., Jordan, S. J., and Mason, R. P. (1990). In vivo thiyl free radical formation from hemoglobin following administration of hydroperoxides. Arch Biochem Biophys *277*, 402-409.

Martin, G. M., Austad, S. N., and Johnson, T. E. (1996). Genetic analysis of ageing: role of oxidative damage and environmental stresses. Nat Genet *13*, 25-34.

Martin, J. L. (1995). Thioredoxin--a fold for all reasons. Structure *3*, 245-250.

Martin, J. L., Waksman, G., Bardwell, J. C., Beckwith, J., and Kuriyan, J. (1993). Crystallization of DsbA, an Escherichia coli protein required for disulphide bond formation in vivo. J Mol Biol *230*, 1097-1100.

McGlynn, P., and Lloyd, R. G. (2000). Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. Cell *101*, 35-45.

McLaggan, D., Logan, T. M., Lynn, D. G., and Epstein, W. (1990). Involvement of gamma-glutamyl peptides in osmoadaptation of Escherichia coli. J Bacteriol *172*, 3631-3636.

Meister, A. (1992). Depletion of glutathione in normal and malignant human cells in vivo by L-buthionine sulfoximine: possible interaction with ascorbate. J Natl Cancer Inst *84*, 1601-1602.

Metheringham, R., Griffiths, L., Crooke, H., Forsythe, S., and Cole, J. (1995). An essential role for DsbA in cytochrome c synthesis and formate- dependent nitrite reduction by Escherichia coli K-12. Arch Microbiol *164*, 301-307.

Metheringham, R., Tyson, K. L., Crooke, H., Missiakas, D., Raina, S., and Cole, J. A. (1996). Effects of mutations in genes for proteins involved in disulphide bond formation in the periplasm on the activities of anaerobically induced electron transfer chains in Escherichia coli K12. Mol Gen Genet *253*, 95-102.

Michan, C., Manchado, M., Dorado, G., and Pueyo, C. (1999). In vivo transcription of the Escherichia coli oxyR regulon as a function of growth phase and in response to oxidative stress. J Bacteriol *181*, 2759-2764.

Minakuchi, K., Yabushita, T., Masumura, T., Ichihara, K., and Tanaka, K. (1994). Cloning and sequence analysis of a cDNA encoding rice glutaredoxin. FEBS Lett *337*, 157-160.

Miranda-Vizuete, A., Damdimopoulos, A. E., Gustafsson, J., and Spyrou, G. (1997). Cloning, expression, and characterization of a novel Escherichia coli thioredoxin. J Biol Chem *272*, 30841-30847.

Miranda-Vizuete, A., Martinez-Galisteo, E., Åslund, F., Lopez-Barea, J., Pueyo, C., and Holmgren, A. (1994). Null thioredoxin and glutaredoxin Escherichia coli K-12

mutants have no enhanced sensitivity to mutagens due to a new GSH-dependent hydrogen donor and high increases in ribonucleotide reductase activity. Journal of Biological Chemistry *269*, 16631-16637.

Miranda-Vizuete, A., Rodriguez-Ariza, A., Toribio, F., Holmgren, A., Lopez-Barea, J., and Pueyo, C. (1996). The levels of ribonucleotide reductase, thioredoxin, glutaredoxin 1, and GSH are balanced in Escherichia coli K12. J Biol Chem *271*, 19099-19103.

Missiakas, D., Georgopoulos, C., and Raina, S. (1994). The Escherichia coli dsbC (xprA) gene encodes a periplasmic protein involved in disulfide bond formation. Embo J *13*, 2013-2020.

Missiakas, D., Georgopoulos, C., and Raina, S. (1993). Identification and characterization of the Escherichia coli gene dsbB, whose product is involved in the formation of disulfide bonds in vivo. Proc Natl Acad Sci U S A *90*, 7084-7088.

Missiakas, D., Schwager, F., and Raina, S. (1995). Identification and characterization of a new disulfide isomerase-like protein (DsbD) in Escherichia coli. Embo J *14*, 3415-3424.

Monika, E. M., Goldman, B. S., Beckman, D. L., and Kranz, R. G. (1997). A thioreduction pathway tethered to the membrane for periplasmic cytochromes c biogenesis; in vitro and in vivo studies. J Mol Biol *271*, 679-692.

Monje-Casas, F., Jurado, J., Prieto-Alamo, M. J., Holmgren, A., and Pueyo, C. (2001). Expression analysis of the nrdHIEF operon from Escherichia coli. Conditions that trigger the transcript level in vivo. J Biol Chem *276*, 18031-18037.

Moore, E. C., Reichard, P., and Thelander, L. (1964). Enzymatic synthesis of deoxiribonucleotides. V. Purification and properties of thioredoxin reductase from Escherichia coli B. J Biol Chem *239*, 3445-3452.

Morell, S., Follmann, H., and Haberlein, I. (1995). Identification and localization of the first glutaredoxin in leaves of a higher plant. FEBS Lett *369*, 149-152.

Morgan, R. W., Christman, M. F., Jacobson, F. S., Storz, G., and Ames, B. N. (1986). Hydrogen peroxide-inducible proteins in Salmonella typhimurium overlap with heat shock and other stress proteins. Proc Natl Acad Sci U S A *83*, 8059-8063.

Mossner, E., Huber-Wunderlich, M., and Glockshuber, R. (1998). Characterization of Escherichia coli thioredoxin variants mimicking the active-sites of other thiol/disulfide oxidoreductases. Protein Sci *7*, 1233-1244.

Mulvey, M. R., and Loewen, P. C. (1989). Nucleotide sequence of katF of Escherichia coli suggests KatF protein is a novel sigma transcription factor. Nucleic Acids Res *17*, 9979-9991.

Mulvey, M. R., Switala, J., Borys, A., and Loewen, P. C. (1990). Regulation of transcription of katE and katF in Escherichia coli. J Bacteriol *172*, 6713-6720.

Nakamura, H., Bai, J., Nishinaka, Y., Ueda, S., Sasada, T., Ohshio, G., Imamura, M., Takabayashi, A., Yamaoka, Y., and Yodoi, J. (2000). Expression of thioredoxin and glutaredoxin, redox-regulating proteins, in pancreatic cancer. Cancer Detect Prev *24*, 53-60.

Nakamura, H., Vaage, J., Valen, G., Padilla, C. A., Bjornstedt, M., and Holmgren, A. (1998). Measurements of plasma glutaredoxin and thioredoxin in healthy volunteers and during open-heart surgery. Free Radic Biol Med *24*, 1176-1186.

Nelson, J. W., and Creighton, T. E. (1994). Reactivity and ionization of the active site cysteine residues of DsbA, a protein required for disulfide bond formation in vivo. Biochemistry *33*, 5974-5983.

Nishida, M., Harada, S., Noguchi, S., Satow, Y., Inoue, H., and Takahashi, K. (1998). Three-dimensional structure of Escherichia coli glutathione S- transferase complexed with glutathione sulfonate: catalytic roles of Cys10 and His106. J Mol Biol *281*, 135-147.

Nishida, M., Kong, K. H., Inoue, H., and Takahashi, K. (1994). Molecular cloning and site-directed mutagenesis of glutathione S- transferase from Escherichia coli. The conserved tyrosyl residue near the N terminus is not essential for catalysis. J Biol Chem *269*, 32536-32541.

Nordstrand, K., Aslund, F., Meunier, S., Holmgren, A., Otting, G., and Berndt, K. D. (1999). Direct NMR observation of the Cys-14 thiol proton of reduced Escherichia coli glutaredoxin-3 supports the presence of an active site thiol-thiolate hydrogen bond. FEBS Lett *449*, 196-200.

Nordstrand, K., slund, F., Holmgren, A., Otting, G., and Berndt, K. D. (1999). NMR structure of Escherichia coli glutaredoxin 3-glutathione mixed disulfide complex: implications for the enzymatic mechanism. J Mol Biol *286*, 541-552.

Nunoshiba, T., Hidalgo, E., Amabile Cuevas, C. F., and Demple, B. (1992). Two-stage control of an oxidative stress regulon: the Escherichia coli SoxR protein triggers redox-inducible expression of the soxS regulatory gene. J Bacteriol *174*, 6054-6060.

Nunoshiba, T., and Yamamoto, K. (1999). Role of glutathione on acrolein-induced cytotoxicity and mutagenicity in Escherichia coli. Mutat Res *442*, 1-8.

Nyström, T. (2002a). Aging in bacteria. Curr Opin Microbiol *5*, 596-601.

Nyström, T. (2001). Not quite dead enough: on bacterial life, culturability, senescence, and death. Arch Microbiol *176*, 159-164.

Nyström, T. (2002b). Translational fidelity, protein oxidation, and senescence: lessons from bacteria. Ageing Res Rev *1*, 693-703.

Okuda, M., Inoue, N., Azumi, H., Seno, T., Sumi, Y., Hirata, K., Kawashima, S., Hayashi, Y., Itoh, H., Yodoi, J., and Yokoyama, M. (2001). Expression of glutaredoxin in human coronary arteries: its potential role in antioxidant protection against atherosclerosis. Arterioscler Thromb Vasc Biol *21*, 1483-1487.

Ollagnier, S., Mulliez, E., Gaillard, J., Eliasson, R., Fontecave, M., and Reichard, P. (1996). The anaerobic Escherichia coli ribonucleotide reductase. Subunit structure and iron sulfur center. J Biol Chem *271*, 9410-9416.

Orr, W. C., and Sohal, R. S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster. Science *263*, 1128-30.

Pace, G. W., and Leaf, C. D. (1995). The role of oxidative stress in HIV disease. Free Radic Biol Med *19*, 523-528.

Padilla, C. A., Martinez-Galisteo, E., Barcena, J. A., Spyrou, G., and Holmgren, A. (1995). Purification from placenta, amino acid sequence, structure comparisons and cDNA cloning of human glutaredoxin. Eur J Biochem *227*, 27-34.

Padilla, C. A., Martinez-Galisteo, E., Lopez-Barea, J., Holmgren, A., and Barcena, J. A. (1992). Immunolocalization of thioredoxin and glutaredoxin in mammalian hypophysis. Mol Cell Endocrinol *85*, 1-12.

Page, M. D., and Ferguson, S. J. (1997). Paracoccus denitrificans CcmG is a periplasmic protein-disulphide oxidoreductase required for c- and aa3-type cytochrome biogenesis; evidence for a reductase role in vivo. Mol Microbiol *24*, 977-990.

Panagou, D., Orr, M. D., Dunstone, J. R., and Blakley, R. L. (1972). A monomeric, allosteric enzyme with a single polypeptide chain. Ribonucleotide reductase of Lactobacillus leichmannii. Biochemistry *11*, 2378-2388.

Penninckx, M. J., and Elskens, M. T. (1993). Metabolism and functions of glutathione in micro-organisms. Adv Microb Physiol *34*, 239-301.

Pianzzola, M. J., Soubes, M., and Touati, D. (1996). Overproduction of the rbo gene product from Desulfovibrio species suppresses all deleterious effects of lack of superoxide dismutase in Escherichia coli. J Bacteriol *178*, 6736-6742.

Pineda-Molina, E., Klatt, P., Vazquez, J., Marina, A., Garcia de Lacoba, M., Perez-Sala, D., and Lamas, S. (2001). Glutathionylation of the p50 subunit of NF-kappaB: a mechanism for redox-induced inhibition of DNA binding. Biochemistry *40*, 14134-14142.

Pomposiello, P. J., and Demple, B. (2001). Redox-operated genetic switches: the SoxR and OxyR transcription factors. Trends Biotechnol *19*, 109-114.

Poole, A. M., Logan, D. T., and Sjoberg, B. M. (2002). The evolution of the ribonucleotide reductases: much ado about oxygen. J Mol Evol *55*, 180-196.

Porras, P., Pedrajas, J. R., Martinez-Galisteo, E., Padilla, C. A., Johansson, C., Holmgren, A., and Barcena, J. A. (2002). Glutaredoxins catalyze the reduction of glutathione by dihydrolipoamide with high efficiency. Biochem Biophys Res Commun *295*, 1046-1051.

Prince, R. W., Xu, Y., Libby, S. J., and Fang, F. C. (1994). Cloning and sequencing of the gene encoding the RpoS (KatF) sigma factor from Salmonella typhimurium 14028s. Biochim Biophys Acta *1219*, 198-200.

Prinz, W. A., Åslund, F., Holmgren, A., and Beckwith, J. (1997). The role of the thioredoxin and glutaredoxin pathways in reducing protein disulfide bonds in the Escherichia coli cytoplasm. J Biol Chem *272*, 15661-15667.

Rahlfs, S., Fischer, M., and Becker, K. (2001). Plasmodium falciparum possesses a classical glutaredoxin and a second, glutaredoxin-like protein with a PICOT homology domain. J Biol Chem *276*, 37133-37140.

Raina, S., and Missiakas, D. (1997). Making and breaking disulfide bonds. Annu Rev Microbiol *51*, 179-202.

Regeimbal, J., and Bardwell, J. C. (2002). DsbB catalyzes disulfide bond formation de novo. J Biol Chem *277*, 32706-32713.

Reichard, P. (1997). The evolution of ribonucleotide reduction. Trends Biochem Sci *22*, 81-85.

Reichard, P. (1993). From RNA to DNA, why so many ribonucleotide reductases? Science *260*, 1773-1777.

Reichard, P. (2002). Ribonucleotide reductases: the evolution of allosteric regulation. Arch Biochem Biophys *397*, 149-155.

Reinemer, P., Dirr, H. W., Ladenstein, R., Schaffer, J., Gallay, O., and Huber, R. (1991). The three-dimensional structure of class pi glutathione S-transferase in complex with glutathione sulfonate at 2.3 A resolution. Embo J *10*, 1997-2005.

Rensing, C., Mitra, B., and Rosen, B. P. (1997). Insertional inactivation of dsbA produces sensitivity to cadmium and zinc in Escherichia coli. J Bacteriol *179*, 2769-2771.

Repetto, M., Reides, C., Gomez Carretero, M. L., Costa, M., Griemberg, G., and Llesuy, S. (1996). Oxidative stress in blood of HIV infected patients. Clin Chim Acta *255*, 107-117.

Rietsch, A., and Beckwith, J. (1998). The genetics of disulfide bond metabolism. Annu Rev Genet *32*, 163-184.

Rietsch, A., Belin, D., Martin, N., and Beckwith, J. (1996). An in vivo pathway for disulfide bond isomerization in Escherichia coli. Proc Natl Acad Sci U S A *93*, 13048-13053.

Rikans, L. E., and Hornbrook, K. R. (1997). Lipid peroxidation, antioxidant protection and aging. Biochim Biophys Acta *1362*, 116-127.

Ritz, D., and Beckwith, J. (2001). Roles of thiol-redox pathways in bacteria. Annu Rev Microbiol *55*, 21-48.

Ritz, D., Lim, J., Reynolds, C. M., Poole, L. B., and Beckwith, J. (2001). Conversion of a peroxiredoxin into a disulfide reductase by a triplet repeat expansion. Science *294*, 158-160.

Ritz, D., Patel, H., Doan, B., Zheng, M., Åslund, F., Storz, G., and Beckwith, J. (2000). Thioredoxin 2 is involved in the oxidative stress response in escherichia coli [In Process Citation]. J Biol Chem *275*, 2505-2512.

Rodriguez-Manzaneque, M. T., Ros, J., Cabiscol, E., Sorribas, A., and Herrero, E. (1999). Grx5 glutaredoxin plays a central role in protection against protein oxidative damage in Saccharomyces cerevisiae. Mol Cell Biol *19*, 8180-8190.

Rodriguez-Manzaneque, M. T., Tamarit, J., Belli, G., Ros, J., and Herrero, E. (2002). Grx5 is a mitochondrial glutaredoxin required for the activity of iron/sulfur enzymes. Mol Biol Cell *13*, 1109-1121.

Rosen, C. F., Poon, R., and Drucker, D. J. (1995). UVB radiation-activated genes induced by transcriptional and posttranscriptional mechanisms in rat keratinocytes. Am J Physiol *268*, C846-C855.

Rossjohn, J., McKinstry, W. J., Oakley, A. J., Verger, D., Flanagan, J., Chelvanayagam, G., Tan, K. L., Board, P. G., and Parker, M. W. (1998). Human theta class glutathione transferase: the crystal structure reveals a sulfate-binding pocket within a buried active site. Structure *6*, 309-322.

Rouhier, N., Gelhaye, E., and Jacquot, J. P. (2002). Exploring the active site of plant glutaredoxin by site-directed mutagenesis. FEBS Lett *511*, 145-149.

Rouhier, N., Gelhaye, E., and Jacquot, J. P. (2002). Glutaredoxin-dependent peroxiredoxin from poplar: protein-protein interaction and catalytic mechanism. J Biol Chem *277*, 13609-13614.

Rouhier, N., Gelhaye, E., Sautiere, P. E., Brun, A., Laurent, P., Tagu, D., Gerard, J., de Fay, E., Meyer, Y., and Jacquot, J. P. (2001). Isolation and characterization of a new peroxiredoxin from poplar sieve tubes that uses either glutaredoxin or thioredoxin as a proton donor. Plant Physiol *127*, 1299-3109.

Rouhier, N., Gelhaye, E., Sautiere, P. E., and Jacquot, J. P. (2002). Enhancement of poplar glutaredoxin expression by optimization of the cDNA sequence. Protein Expr Purif *24*, 234-241.

Rozell, B., Barcena, J. A., Martinez-Galisteo, E., Padilla, C. A., and Holmgren, A. (1993). Immunochemical characterization and tissue distribution of glutaredoxin (thioltransferase) from calf. Eur J Cell Biol *62*, 314-323.

Russel, M., and Holmgren, A. (1988). Construction and characterization of glutaredoxin-negative mutants of Escherichia coli. Proc Natl Acad Sci U S A *85*, 990-994.

Russel, M., and Model, P. (1985). Thioredoxin is required for filamentous phage assembly. Proc Natl Acad Sci U S A *82*, 29-33.

Russel, M., Model, P., and Holmgren, A. (1990). Thioredoxin or glutaredoxin in Escherichia coli is essential for sulfate reduction but not for deoxyribonucleotide synthesis. J Bacteriol *172*, 1923-1929.

Sahlin, L., Ostlund, E., Wang, H., Holmgren, A., and Fried, G. (2000). Decreased expression of thioredoxin and glutaredoxin in placentae from pregnancies with pre-eclampsia and intrauterine growth restriction. Placenta *21*, 603-609.

Sahlin, L., Wang, H., Stjernholm, Y., Lundberg, M., Ekman, G., Holmgren, A., and Eriksson, H. (2000). The expression of glutaredoxin is increased in the human cervix in term pregnancy and immediately post-partum, particularly after prostaglandin- induced delivery. Mol Hum Reprod *6*, 1147-1153.

Saint, N., Lacapere, J. J., Gu, L. Q., Ghazi, A., Martinac, B., and Rigaud, J. L. (1998). A hexameric transmembrane pore revealed by two-dimensional crystallization of the large mechanosensitive ion channel (MscL) of Escherichia coli. J Biol Chem *273*, 14667-14670.

Sambongi, Y., and Ferguson, S. J. (1996). Mutants of Escherichia coli lacking disulphide oxidoreductases DsbA and DsbB cannot synthesise an exogenous monohaem c-type cytochrome except in the presence of disulphide compounds. FEBS Lett *398*, 265-268.

Sandberg, V. A., Kren, B., Fuchs, J. A., and Woodward, C. (1991). Escherichia coli glutaredoxin: cloning and overexpression, thermodynamic stability of the oxidized and reduced forms, and report of an N-terminal extended species. Biochemistry *30*, 5475-5484.

Savage, H., Montoya, G., Svensson, C., Schwenn, J. D., and Sinning, I. (1997). Crystal structure of phosphoadenylyl sulphate (PAPS) reductase: a new family of adenine nucleotide alpha hydrolases. Structure *5*, 895-906.

Schafer, F. Q., and Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med *30*, 1191-1212.

Scharf, C., Riethdorf, S., Ernst, H., Engelmann, S., Volker, U., and Hecker, M. (1998). Thioredoxin is an essential protein induced by multiple stresses in Bacillus subtilis. J Bacteriol *180*, 1869-1877.

Schweder, T., Lee, K. H., Lomovskaya, O., and Matin, A. (1996). Regulation of Escherichia coli starvation sigma factor (sigma s) by ClpXP protease. J Bacteriol *178*, 470-476.

Sheehan, D., Meade, G., Foley, V. M., and Dowd, C. A. (2001). Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J *360*, 1-16.

Shi, J., Vlamis-Gardikas, A., Åslund, F., Holmgren, A., and Rosen, B. P. (1999). Reactivity of glutaredoxins 1, 2, and 3 from Escherichia coli shows that glutaredoxin 2 is the primary hydrogen donor to ArsC-catalyzed arsenate reduction. J Biol Chem *274*, 36039-36042.

Shin, S., Castanie-Cornet, M. P., Foster, J. W., Crawford, J. A., Brinkley, C., and Kaper, J. B. (2001). An activator of glutamate decarboxylase genes regulates the expression of enteropathogenic Escherichia coli virulence genes through control of the plasmid-encoded regulator, Per. Mol Microbiol *41*, 1133-1150.

Sinha, R. P. (1986). Toxicity of organic acids for repair-deficient strains of Escherichia coli. Appl Environ Microbiol *51*, 1364-1366.

Sjöberg, B.-M. (1997). Ribonucleotide reductase-A group of enzymes with different metallosites and similar reaction mechanism. Struct Bond *88*, 139-173.

Sjöberg, B. M., and Holmgren, A. (1972). Studies on the structure of T4 thioredoxin. II. Amino acid sequence of the protein and comparison with thioredoxin from Escherichia coli. J Biol Chem *247*, 8063-8068.

Sjöberg, B. M., and Sahlin, M. (2002). Thiols in redox mechanism of ribonucleotide reductase. Methods Enzymol *348*, 1-21.

Small, P., Blankenhorn, D., Welty, D., Zinser, E., and Slonczewski, J. L. (1994). Acid and base resistance in Escherichia coli and Shigella flexneri: role of rpoS and growth pH. J Bacteriol *176*, 1729-1737.

Sodano, P., Chary, K. V., Bjornberg, O., Holmgren, A., Kren, B., Fuchs, J. A., and Wuthrich, K. (1991). Nuclear magnetic resonance studies of recombinant Escherichia coli glutaredoxin. Sequence-specific assignments and secondary structure determination of the oxidized form. Eur J Biochem *200*, 369-377.

Sodano, P., Xia, T. H., Bushweller, J. H., Bjornberg, O., Holmgren, A., Billeter, M., and Wuthrich, K. (1991). Sequence-specific 1H n.m.r. assignments and determination of the three- dimensional structure of reduced Escherichia coli glutaredoxin. J Mol Biol *221*, 1311-1324.

Sohal, R. S., and Weindruch, R. (1996). Oxidative stress, caloric restriction, and aging. Science *273*, 59-63.

Spina, M. B., and Cohen, G. (1989). Dopamine turnover and glutathione oxidation: implications for Parkinson disease. Proc Natl Acad Sci U S A *86*, 1398-1400.

Staal, F. J., Roederer, M., and Herzenberg, L. A. (1992). Glutathione and immunophenotypes of T and B lymphocytes in HIV-infected individuals. Ann N Y Acad Sci *651*, 453-463.

Stafford, S. J., Humphreys, D. P., and Lund, P. A. (1999). Mutations in dsbA and dsbB, but not dsbC, lead to an enhanced sensitivity of Escherichia coli to Hg2+ and Cd2+. FEMS Microbiol Lett *174*, 179-184.

Stavreus-Evers, A., Masironi, B., Landgren, B. M., Holmgren, A., Eriksson, H., and Sahlin, L. (2002). Immunohistochemical localization of glutaredoxin and thioredoxin in human endometrium: a possible association with pinopodes. Mol Hum Reprod *8*, 546-551.

Stehr, M., Schneider, G., Aslund, F., Holmgren, A., and Lindqvist, Y. (2001). Structural basis for the thioredoxin-like activity profile of the glutaredoxin-like NrdH-redoxin from Escherichia coli. J Biol Chem *276*, 35836-35841.

Stewart, E. J., Åslund, F., and Beckwith, J. (1998). Disulfide bond formation in the Escherichia coli cytoplasm: an in vivo role reversal for the thioredoxins. Embo J *17*, 5543-5550.

Stewart, E. J., Katzen, F., and Beckwith, J. (1999). Six conserved cysteines of the membrane protein DsbD are required for the transfer of electrons from the cytoplasm to the periplasm of Escherichia coli. Embo J *18*, 5963-5971.

Storz, G., and Imlay, J. A. (1999). Oxidative stress. Curr Opin Microbiol *2*, 188-94.

Storz, G., Jacobson, F. S., Tartaglia, L. A., Morgan, R. W., Silveira, L. A., and Ames, B. N. (1989). An alkyl hydroperoxide reductase induced by oxidative stress in Salmonella typhimurium and Escherichia coli: genetic characterization and cloning of ahp. J Bacteriol *171*, 2049-2055.

Storz, G., and Tartaglia, L. A. (1992). OxyR: a regulator of antioxidant genes. J Nutr *122*, 627-630.

Storz, G., Tartaglia, L. A., and Ames, B. N. (1990). Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science *248*, 189-194.

Sukharev, S. I., Schroeder, M. J., and McCaslin, D. R. (1999). Stoichiometry of the large conductance bacterial mechanosensitive channel of E. coli. A biochemical study. J Membr Biol *171*, 183-193.

Sun, C., Berardi, M. J., and Bushweller, J. H. (1998). The NMR solution structure of human glutaredoxin in the fully reduced form. J Mol Biol *280*, 687-701.

Sun, Q. A., Kirnarsky, L., Sherman, S., and Gladyshev, V. N. (2001). Selenoprotein oxidoreductase with specificity for thioredoxin and glutathione systems. Proc Natl Acad Sci U S A *98*, 3673-3678.

Sun, X., Ollagnier, S., Schmidt, P. P., Atta, M., Mulliez, E., Lepape, L., Eliasson, R., Graslund, A., Fontecave, M., Reichard, P., and Sjoberg, B. M. (1996). The free radical of the anaerobic ribonucleotide reductase from Escherichia coli is at glycine 681. J Biol Chem *271*, 6827-6831.

Tamarit, J., Mulliez, E., Meier, C., Trautwein, A., and Fontecave, M. (1999). The anaerobic ribonucleotide reductase from Escherichia coli. The small protein is an activating enzyme containing a [4fe-4s](2+) center. J Biol Chem *274*, 31291-31296.

Tao, K. (1997). oxyR-dependent induction of Escherichia coli grx gene expression by peroxide stress. J Bacteriol *179*, 5967-5970.

Tartaglia, L. A., Storz, G., and Ames, B. N. (1989). Identification and molecular analysis of oxyR-regulated promoters important for the bacterial adaptation to oxidative stress. J Mol Biol *210*, 709-719.

Thelander, L. (1967). Thioredoxin reductase. Characterization of a homogenous preparation from Escherichia coli B. J Biol Chem *242*, 852-859.

Thony-Meyer, L. (2002). Cytochrome c maturation: a complex pathway for a simple task? Biochem Soc Trans *30*, 633-638.

Toledano, M. B., Kullik, I., Trinh, F., Baird, P. T., Schneider, T. D., and Storz, G. (1994). Redox-dependent shift of OxyR-DNA contacts along an extended DNA-binding site: a mechanism for differential promoter selection. Cell *78*, 897-909.

Torrents, E., Jordan, A., Karlsson, M., and Gibert, I. (2000). Occurrence of multiple ribonucleotide reductase classes in gamma- proteobacteria species. Curr Microbiol *41*, 346-351.

Triggs-Raine, B. L., Doble, B. W., Mulvey, M. R., Sorby, P. A., and Loewen, P. C. (1988). Nucleotide sequence of katG, encoding catalase HPI of Escherichia coli. J Bacteriol *170*, 4415-4419.

Triggs-Raine, B. L., and Loewen, P. C. (1987). Physical characterization of katG, encoding catalase HPI of Escherichia coli. Gene *52*, 121-128.

Tsai, P. K., and Hogenkamp, H. P. (1980). The purification and characterization of an adenosylcobalamin-dependent ribonucleoside diphosphate reductase from Corynebacterium nephridii. J Biol Chem *255*, 1273-1278.

Tsaneva, I. R., and Weiss, B. (1990). soxR, a locus governing a superoxide response regulon in Escherichia coli K-12. J Bacteriol *172*, 4197-4205.

Tsang, M. L. (1981). Assimilatory sulfate reduction in Escherichia coli: identification of the alternate cofactor for adenosine 3'-phosphate 5'-phosphosulfate reductase as glutaredoxin. J Bacteriol *146*, 1059-1066.

Tsang, M. L., and Schiff, J. A. (1978). Assimilatory sulfate reduction in an Escherichia coli mutant lacking thioredoxin activity. J Bacteriol *134*, 131-138.

Tsang, M. L., and Schiff, J. A. (1976). Sulfate-reducing pathway in Escherichia coli involving bound intermediates. J Bacteriol *125*, 923-933.

Tuggle, C. K., and Fuchs, J. A. (1985). Glutathione reductase is not required for maintenance of reduced glutathione in Escherichia coli K-12. J Bacteriol *162*, 448-50.

Vlamis-Gardikas, A., Åslund, F., Spyrou, G., Bergman, T., and Holmgren, A. (1997). Cloning, overexpression, and characterization of glutaredoxin 2, an atypical glutaredoxin from Escherichia coli. J Biol Chem *272*, 11236-11243.

Vlamis-Gardikas, A., and Holmgren, A. (2002). Thioredoxin and glutaredoxin isoforms. Methods Enzymol *347*, 286-296.

Vuilleumier, S. (1997). Bacterial glutathione S-transferases: what are they good for? J Bacteriol *179*, 1431-1441.

Wang, J., Boja, E. S., Tan, W., Tekle, E., Fales, H. M., English, S., Mieyal, J. J., and Chock, P. B. (2001). Reversible glutathionylation regulates actin polymerization in A431 cells. J Biol Chem *276*, 47763-47766.

Waterman, S. R., and Small, P. L. (1996). Identification of sigma S-dependent genes associated with the stationary-phase acid-resistance phenotype of Shigella flexneri. Mol Microbiol *21*, 925-940.

Webb, C., Moreno, M., Wilmes-Riesenberg, M., Curtiss, R., 3rd, and Foster, J. W. (1999). Effects of DksA and ClpP protease on sigma S production and virulence in Salmonella typhimurium. Mol Microbiol *34*, 112-123.

Wells, W. W., Rocque, P. A., Xu, D. P., Meyer, E. B., Charamella, L. J., and Dimitrov, N. V. (1995). Ascorbic acid and cell survival of adriamycin resistant and sensitive MCF-7 breast tumor cells. Free Radic Biol Med *18*, 699-708.

Wells, W. W., Rocque, P. A., Xu, D. P., Yang, Y., and Deits, T. L. (1991). Interactions of platinum complexes with thioltransferase(glutaredoxin), in vitro. Biochem Biophys Res Commun *180*, 735-741.

Wells, W. W., Xu, D. P., Yang, Y. F., and Rocque, P. A. (1990). Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. J Biol Chem *265*, 15361-15364.

White, C. L., Senkevich, T. G., and Moss, B. (2002). Vaccinia virus G4L glutaredoxin is an essential intermediate of a cytoplasmic disulfide bond pathway required for virion assembly. J Virol *76*, 467-472.

Williams, C. H., Arscott, L. D., Muller, S., Lennon, B. W., Ludwig, M. L., Wang, P. F., Veine, D. M., Becker, K., and Schirmer, R. H. (2000). Thioredoxin reductase two modes of catalysis have evolved. Eur J Biochem *267*, 6110-6117.

Witte, S., Villalba, M., Bi, K., Liu, Y., Isakov, N., and Altman, A. (2000). Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-kappaB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. J Biol Chem *275*, 1902-1909.

Wood, J. M. (1999). Osmosensing by bacteria: signals and membrane-based sensors. Microbiol Mol Biol Rev *63*, 230-262.

Wu, J., Dunham, W. R., and Weiss, B. (1995). Overproduction and physical characterization of SoxR, a [2Fe-2S] protein that governs an oxidative response regulon in Escherichia coli. J Biol Chem *270*, 10323-10327.

Wu, J., and Weiss, B. (1991). Two divergently transcribed genes, soxR and soxS, control a superoxide response regulon of Escherichia coli. J Bacteriol *173*, 2864-2871.

Wu, J., and Weiss, B. (1992). Two-stage induction of the soxRS (superoxide response) regulon of Escherichia coli. J Bacteriol *174*, 3915-3920.

Wunderlich, M., and Glockshuber, R. (1993). Redox properties of protein disulfide isomerase (DsbA) from Escherichia coli. Protein Sci *2*, 717-726.

Xia, B., Vlamis-Gardikas, A., Holmgren, A., Wright, P. E., and Dyson, H. J. (2001). Solution structure of Escherichia coli glutaredoxin-2 shows similarity to mammalian glutathione-S-transferases. J Mol Biol *310*, 907-918.

Xia, T. H., Bushweller, J. H., Sodano, P., Billeter, M., Bjornberg, O., Holmgren, A., and Wuthrich, K. (1992). NMR structure of oxidized Escherichia coli glutaredoxin: comparison with reduced E. coli glutaredoxin and functionally related proteins. Protein Sci *1*, 310-321.

Xiao, H., Kalman, M., Ikehara, K., Zemel, S., Glaser, G., and Cashel, M. (1991). Residual guanosine 3',5'-bispyrophosphate synthetic activity of relA null mutants can be eliminated by spoT null mutations. J Biol Chem *266*, 5980-5990.

Yamanaka, H., Kameyama, M., Baba, T., Fujii, Y., and Okamoto, K. (1994). Maturation pathway of Escherichia coli heat-stable enterotoxin I: requirement of DsbA for disulfide bond formation. J Bacteriol *176*, 2906-2913.

Yang, Y. F., and Wells, W. W. (1991). Identification and characterization of the functional amino acids at the active center of pig liver thioltransferase by site-directed mutagenesis. J Biol Chem *266*, 12759-12765.

Zgurskaya, H. I., Keyhan, M., and Matin, A. (1997). The sigma S level in starving Escherichia coli cells increases solely as a result of its increased stability, despite decreased synthesis. Mol Microbiol *24*, 643-651.

Zheng, M., Aslund, F., and Storz, G. (1998). Activation of the OxyR transcription factor by reversible disulfide bond formation [see comments]. Science *279*, 1718-1721.

Zheng, M., Wang, X., Templeton, L. J., Smulski, D. R., LaRossa, R. A., and Storz, G. (2001). DNA microarray-mediated transcriptional profiling of the Escherichia coli response to hydrogen peroxide. J Bacteriol *183*, 4562-4570.

Zhong, L., Arn-er, E. S., Ljung, J., Aslund, F., and Holmgren, A. (1998). Rat and calf thioredoxin reductase are homologous to glutathione reductase with a carboxyl-terminal elongation containing a conserved catalytically active penultimate selenocysteine residue. J Biol Chem *273*, 8581-8591.

Zhong, L., and Holmgren, A. (2000). Essential role of selenium in the catalytic activities of mammalian thioredoxin reductase revealed by characterization of recombinant enzymes with selenocysteine mutations. J Biol Chem *275*, 18121-1812
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